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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1973

J. D. MacEwen, et al

California University

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UNIVERSITY OF CALIFORNIA

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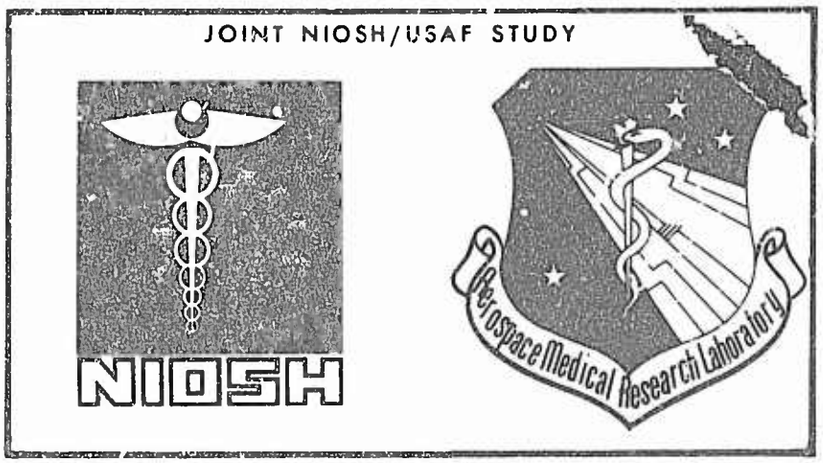

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13. ABSTRACT

The activities of the Toxic Hazards Research Unit (THRU) for the period of June 1972 through May 1973 are reviewed in this report. Acute inhalation toxicity experiments were conducted on mixtures of carbon monoxide and hydrogen cyanide. Emergency Exposure Limits (EEL) were validated for chlorine pentafluoride and a 24-hour EEL defined for monomethylhydrazine. A series of chronic inhalation toxicity studies were conducted with hydrazine, methylhydrazine and coal tar aerosols. Oral percutaneous toxicity evaluations were conducted on a series of chemical agents.

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Percutaneous Toxicity						
Instrumentation						
Catalytic Heater Testing						

FOREWORD

This is the ninth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the University of California on behalf of the Air Force under Contract No. F33615-73-C-4059. This constitutes the first report under the current contract and describes the accomplishments of the THRU from June 1972 through May 1973. Part of the work described was performed under Contract No. F33615-70-C-1046 with SysteMed Corporation using the same THRU staff.

The current contract for operation of the Laboratory was initiated in 1972 under Project 6302 "Toxic Hazards of Propellants and Materials," Task 01 "Toxicology" Work Unit No. 63020113. K. C. Back, PhD, Chief of the Toxicology Branch, was the technical contract monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, PhD, served as co-principal investigator and Laboratory Director for the THRU with SysteMed Corporation and the University of California. Acknowledgement is made to C. E Johnson, C. C. Haun, G. L. Fogle and J. H. Archibald for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the Department of Transportation and the National Institute of Occupational Safety and Health.

This technical report has been reviewed and is approved.

ANTHONY A. THOMAS, MD
Director
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SECTION I

INTRODUCTION

This document constitutes the 9th annual report of the Toxic Hazards Research Unit, (THRU), a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the Air Force and other governmental agencies. The THRU research team is an interdisciplinary group of toxicologists, chemists, statisticians, and engineers supported by Air Force pathologists, veterinarians, and medical technologists.

With the comprehensive scientific team and exposure resources described above the THRU can conduct realistic simulation of human exposures to contaminants causing adverse health effects. These exposures, provided to multiple animal species, are carefully monitored using continuous analytical techniques. The animals used in the experimental programs are also monitored by continual visual observation and regularly scheduled biochemical and physiological measurements.

During the first six years of operation, the primary research efforts of the THRU were directed to obtaining information on health hazards of spacecraft flight, and the biological data obtained have been used as criteria for setting continuous exposure limits and for engineering design factors. This research effort is continuing on a lesser scale while more emphasis has been placed on obtaining data useful for solution of problems of military or civil aircraft emergencies,

community emergencies, and chronic industrial exposures. To this end many of the current research programs serve the mutual interest of the Air Force and other governmental agencies such as the National Institute of Occupational Safety and Health, the Department of Transportation and its Federal Aviation Agency.

The research facilities used by the THRU consist of animal exposure chambers and supporting laboratories. The chamber facilities consist of three types, each performing a separate function. Preconditioning chambers are used to prepare and stabilize animals in a controlled environment. Rochester and Longley Chambers are used for exposing animals to airborne contaminants under ambient conditions of pressure and air composition. These ambient chambers are useful for acute inhalation exposures as well as intermittent long-term chronic exposure experiments. Eight unique and extremely versatile altitude chambers (designated herein as Thomas Domes) are used for conducting long term continuous or intermittent subacute and chronic exposure studies. These Thomas Domes are capable of operating at absolute pressures ranging from 260 to 760 torr utilizing gas mixtures ranging from 20 to 100% oxygen and 0-80% of a secondary gas or mixture of gases. Environmental control of relative humidity, temperature, pressure, and gas flow rate is very stable and precise through continuous monitoring and feedback modulation of regulating valves. The control equipment is provided in replicate and failsafe form so that uninterrupted exposures may be conducted for indefinite periods. More detailed description of the design and operation of the THRU facility is published (Fairchild, 1967; MacEwen, 1965; MacEwen and Geckler, 1966; MacEwen and Vernot, 1968, 1969, 1970; Thomas, 1968).

As part of its contract responsibilities, THRU presents an annual technical conference to disseminate new toxicological information to Air Force, other governmental and industrial scientists. This year's conference presented 22 technical papers and had as a central theme "Biological Threshold Limits" with emphasis on their application in environmental control as a supplement to air quality monitoring. The open forum discussion following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 25 October through 27 October 1972, drew 120 participants including speakers.

During the past year the THRU contract expired and a new 3-year contract was awarded to the Department of Community and Environmental Medicine of the University of California Irvine (UCI) on December 1, 1972. The entire THRU staff was retained by UCI and the new contract without interruption of research programs in progress.

As part of the contractual agreement the University of California has acquired the privilege of utilizing 20% of the THRU facilities for University research programs. To strengthen the research programs of THRU a Scientific Management Team was formed. This team was drawn University wide and represents the professional fields of occupational medicine, pharmacology, pathology, industrial hygiene, neuropharmacology, physiology, biochemistry, management sciences, and biostatistics. The Scientific Management Team meets approximately 3 to 4 times per year. Monthly communication provides information on the progress of THRU programs and new research protocols for review. The functions of the team are as follows:

I. To review recent, current, and planned research and to provide a critique thereof.

A. To improve the basic research protocol, improving the quality and broadening the scope of the research conducted in the Division of Environmental Toxicology (UCI and THRU).

B. To identify opportunities for related research which will benefit the current programs.

C. To identify outside sources of support with interests common to Division programs.

D. To assist in the development and recruiting of the scientific staff.

II. To develop educational activities and research programs in behalf of the organizations to which the members of the advisory team belong.

A. To develop and coordinate the inter-campus joint degree program for graduate students.

B. To conduct "piggy-back" projects of interest to members and/or their associates.

C. To broaden the research capabilities and facilities available to individual members for use in their own research programs and activities.

The first formal meeting of the Scientific Management Team was held at Wright-Patterson Air Force Base, 29-30 March 1973. This meeting was used to familiarize the team members with the scope of research performed at THRU, its capabilities and research facilities.

SECTION II

RESEARCH PROGRAM

The research function of the THRU is a continuing activity which has been conducted independent of contract years or contractors. Experiments are usually in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports are only summarized in this document. This year's research program was conducted on a broader range of materials than previously and includes inhalation studies of propellant oxidizers and fuels, organic solvents, coke oven effluents, irritant gases, and aircraft cabin material pyrolysis products. Oral and percutaneous toxicity investigations on commercial chemicals to which the general public may be exposed through transportation accidents were also conducted.

Effects of 90-Day Continuous Low Level Exposures to Monomethylhydrazine (MMH) on Animals

Monomethylhydrazine has been shown to cause a dose related hemolytic anemia in animals exposed either continuously or intermittently to low atmospheric concentrations of this highly reactive chemical. The chronic effects of MMH exposure have been described by MacEwen and Haun (1971). Dogs, monkeys, rats, and mice were exposed to MMH to evaluate the degree of safety of the current industrial threshold limit (TLV) of 0.2 ppm which was established by analogy to hydrazine and UDMH chronic toxicity. Exposures were conducted

6 hours daily for a 6-month period at MMH concentrations of 0.2, 1.0, 2.0, and 5.0 ppm. These experiments showed dose related growth depression in rats and a linear dose response of hemolytic effects in dogs and monkeys. The hemolytic response appeared to result from the reaction between MMH and hemoglobin to form methemoglobin. In acute exposures the methemoglobin formed is rapidly converted back to oxygemoglobin while under chronic exposure conditions the methemoglobinemia reaches an equilibrium level for each exposure concentration and is accompanied by Heinz body formation. A dose related response of depressed red blood cell count, hematocrit and total hemoglobin were also observed along with increased reticulocytes, serum alkaline phosphatase and serum bilirubin levels. A further indication of the MMH induced changes was a dose related depression of the ratio of myeloid to erythroid elements of bone marrow. No threshold effect level was observed at the MMH concentrations tested.

Animals were also continuously exposed to 0.2 ppm MMH for a 6-month period and the observed effect of hemolytic changes was comparable with an intermittent exposure to 1.0 ppm MMH. These two exposure conditions were equivalent weekly doses in terms of part per million hours (ppm hrs).

One of the major toxic effects of MMH in dogs is the production of severe renal damage. After exposure to subconvulsive levels of MMH, dogs exhibited hematuria, hemoglobinuria, methemoglobinuria, and cast formation. Histopathological examination of the kidneys revealed proteinaceous precipitates in the proximal tubules with occasional hemoglobin casts, moderate to severe degeneration of the proximal tubules with actual tubular necrosis present in many

cases (Sopher 1967). Van Stee (1965) reported a decrease in tubular excretion in dogs following MMH exposure; there was also a decrease in glomerular filtration rate, which he attributed to a decrease in the renal plasma flow rate. Renal function was not affected by MMH in monkeys but subcellular morphologic kidney changes were described by George et al. (1968). Kroe (1971) found periportal choleostasis, bile duct proliferation and hemosiderosis in livers of mice and dogs after long-term chronic exposure to MMH. He also observed renal tubular hemosiderosis in both dogs and mice. These effects were not seen at exposure concentrations below 1 ppm MMH in air.

A need arose for additional information about the effect of continuous exposure to MMH in confined spaces in order to establish specific safety standards for these conditions. The research program described in this report was designed to extend the previous continuous exposure studies to a level of insignificant effect on red blood cells. For this purpose atmospheric concentrations of 0.1 and 0.04 ppm MMH were selected and groups of dogs, monkeys and rats were continuously exposed for 90 days in Thomas Dome chambers. Control groups of unexposed animals were maintained under identical environmental conditions in another Thomas Dome.

Each experimental group of animals, including the control, consisted of 8 female beagle dogs, 4 female rhesus monkeys and 80 male albino rats (Sprague-Dawley strain CFE). All animals were weighed biweekly throughout the study.

At 45 and 90 days of exposure 30 rats from each group were killed and samples taken for hematology measurements. Gross pathology observations were made and tissue specimens were collected for histologic evaluation by others.

Based on the finding from previous MMH exposures a battery of tests was selected for evaluation of the continuous MMH exposures. Dogs and monkeys were tested as shown in Table 1.

TABLE 1. CLINICAL TEST SCHEDULE FOR ANIMALS EXPOSED TO MMH

Test	0	2	4	6	8	10	12	13
HCT	x	x	x	x	x	x	x	x
HGB	x	x	x	x	x	x	x	x
RBC	x	x	x	x	x	x	x	x
WBC	x	x	x	x	x	x	x	x
Total Inorganic Phosphorus	x	x	x	x	x	x	x	x
Alkaline Phosphatase	x	x	x	x	x	x	x	x
Reticulocytes	x	x	x	x	x	x	x	x
Heinz Bodies	x	x	x	x	x	x	x	x
Body Weights (rats also)	x	x	x	x	x	x	x	x
RBC Fragility (dogs only)								x
Gross and Histopathology								x

The MMH introduction system is shown schematically in Figure 1. Liquid MMH was expressed from a 20 ml glass syringe mounted on a single syringe feeder at a constant rate into a heated stainless steel tube containing a flow of 1 liter/minute dry nitrogen. The nitrogen stream with the MMH vapor was then split three ways, one for each of the two exposure domes, and one for venting the excess through a water scrubber. The nitrogen-MMH flow through each of these three paths was controlled by stainless steel valves and flowmeters. The contaminant flow lines then joined the chamber input air prior to entry in the domes. The syringe pump apparatus and all of the valves and flowmeters were housed in a fume hood for safety.

The method of MMH analysis used in this study was a modification of the method reported by Geiger and Vernot (1967). The analytical system is shown schematically in Figure 2. A 2 liter/minute sample was drawn from the dome exhaust line through a teflon line to the top of a glass scrubber column which was filled with glass beads to increase the surface area for mixing the MMH vapor with the absorber solution. The absorber solution was prepared from distilled water by mixing 40 g/liter KI to prevent iodine volatilization, 20 g/liter Na_2HPO_4 and 6 g/liter KH_2PO_4 to maintain the buffer at pH 7.

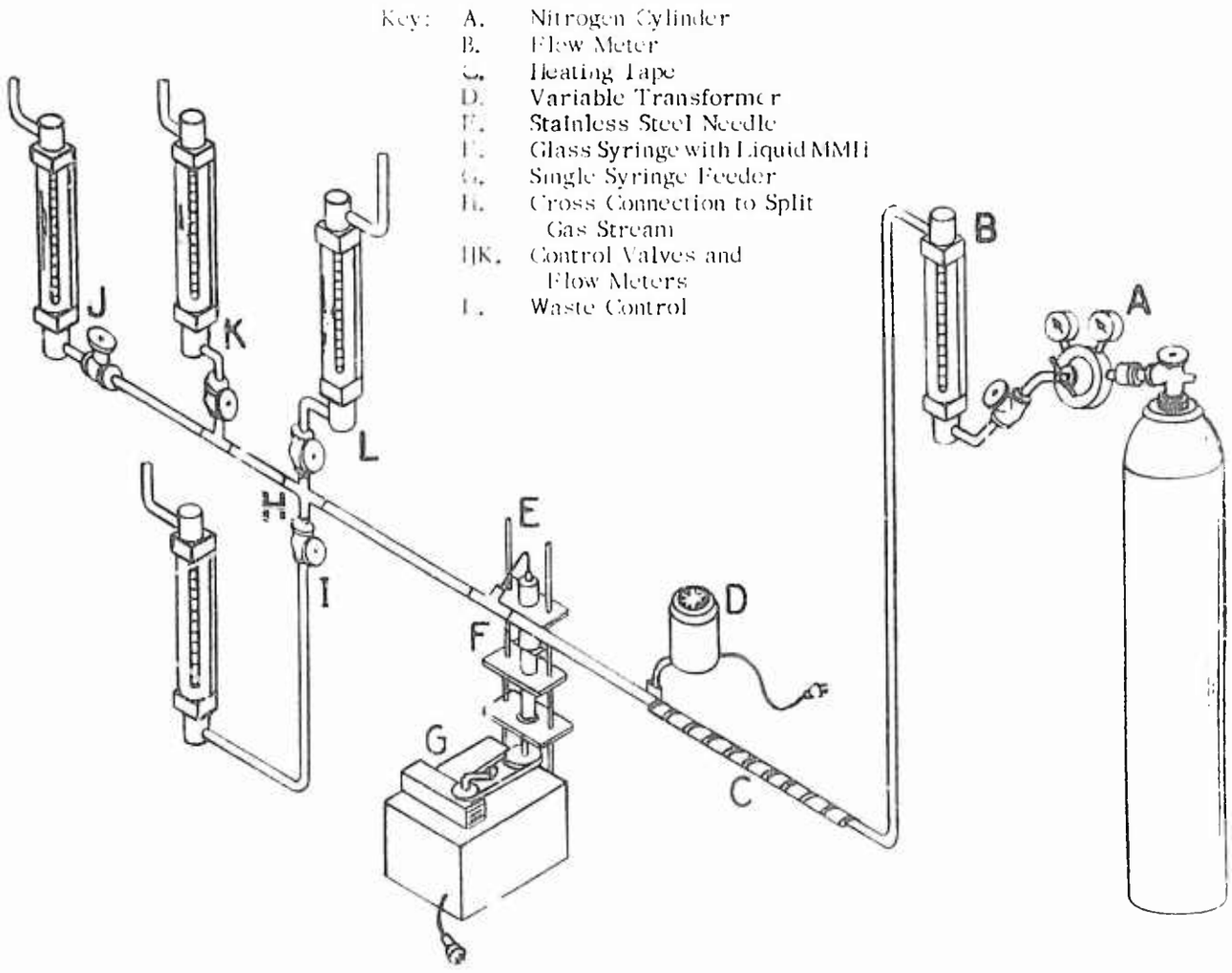
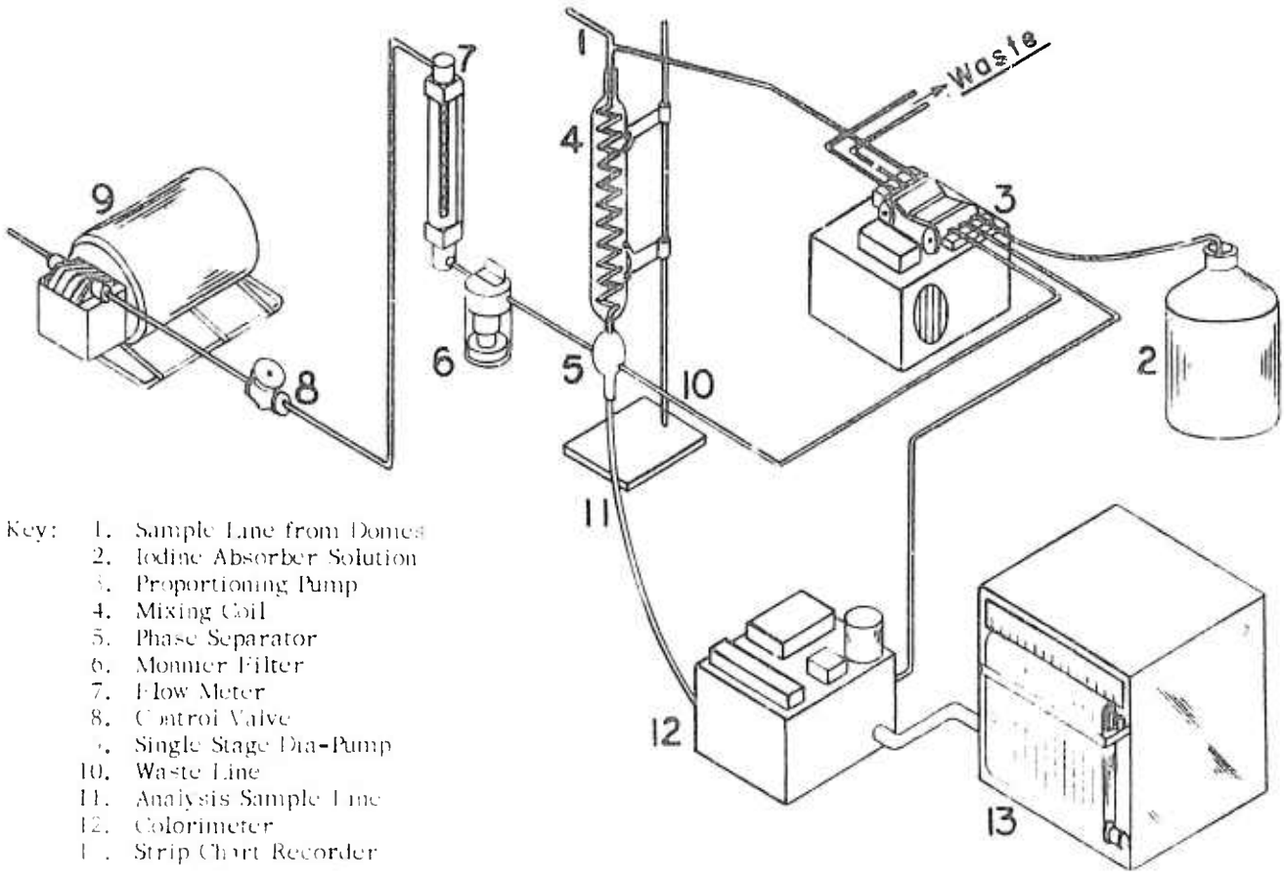


Figure 1. Introduction system for MMH exposures.



Key:
1. Sample Line from Domes
2. Iodine Absorber Solution
3. Proportioning Pump
4. Mixing Coil
5. Phase Separator
6. Monnier Filter
7. Flow Meter
8. Control Valve
9. Single Stage Dia-Pump
10. Waste Line
11. Analysis Sample Line
12. Colorimeter
13. Strip Chart Recorder

Figure 2. Monomethylhydrazine analytical system.

While passing through the column, the MMH dissolved in the liquid and reacted with the iodine, in a colorimetric reaction. The air and liquid were then separated in a glass separator, with the air being drawn through a Monnier filter to remove any water, and then through a flow meter and out through the vacuum pump. The buffer, containing the reacted MMH, was then pumped to a Technicon AutoAnalyzer colorimeter to determine the amount of MMH present in the sample by comparison to a standard having a known amount of MMH. The results were recorded continuously on a strip chart recorder.

The analysis was calibrated with 200 liter mylar bag standards prepared by vaporizing liquid MMH slowly from a needle into dry air. The standard curve was always a straight line through the origin for concentration versus absorbance. The control dome was also sampled throughout the study as a baseline for the two experimental domes, since the animals in a normal dome load give certain known and unknown products which cause minor reaction with the iodine solution. Each of the three domes was sampled sequentially for 40 minutes around the clock, giving a 2-hour cycle for monitoring all three domes.

The nominal 0.10 ppm MMH exposure chamber had a 90-day mean concentration of 0.10 ppm with 24 hour means ranging from 0.08 ppm to 0.12 ppm and the 0.04 ppm MMH exposure resulted in a 90-day mean concentration of 0.04 ppm MMH with a range of 0.03 ppm to 0.05 ppm for individual daily mean concentrations.

Animals were fed ad libitum during exposure and all old food was removed during the daily chamber cleaning period.

The continuous 90-day exposure of animals to an atmospheric MMH concentration of 0.1 ppm produced measurable effects in dogs and rats. Rat growth rate was depressed as shown in Figure 3 and the difference between the exposed group and the controls was significant at the 0.01 level until the last weighing period. Rat organ weights and organ to body weight ratios are shown in Table 2. Rat hematology values were slightly lower in both exposure groups as seen in Table 3, suggesting some hemolytic effect. This change was statistically significant after 45 days of exposure but was not significant at 90 days, although still present. Gross pathology examinations conducted on rats failed to show any significant differences.

Beagle dogs showed significant increases in serum phosphorus and alkaline phosphatase levels during the exposure period as shown in Tables 4 and 5. Hematologic changes were seen only at the 0.1 ppm MMH concentration where significant hemolytic effects were noted as shown in Table 6 which presents the terminal blood measurements for dogs and monkeys. The red blood cells of the dogs exposed at the 0.1 ppm level demonstrated increased osmotic fragility when compared with controls. No significant change occurred at the 0.04 ppm level for this test. Gross pathologic changes were observed at the highest concentration (0.1 ppm MMH) only in dogs. The livers of the exposed dogs had a nutmeg appearance consistent with the passive congestion previously seen at higher dose levels. There were no gross differences between control and the MMH exposed monkeys.

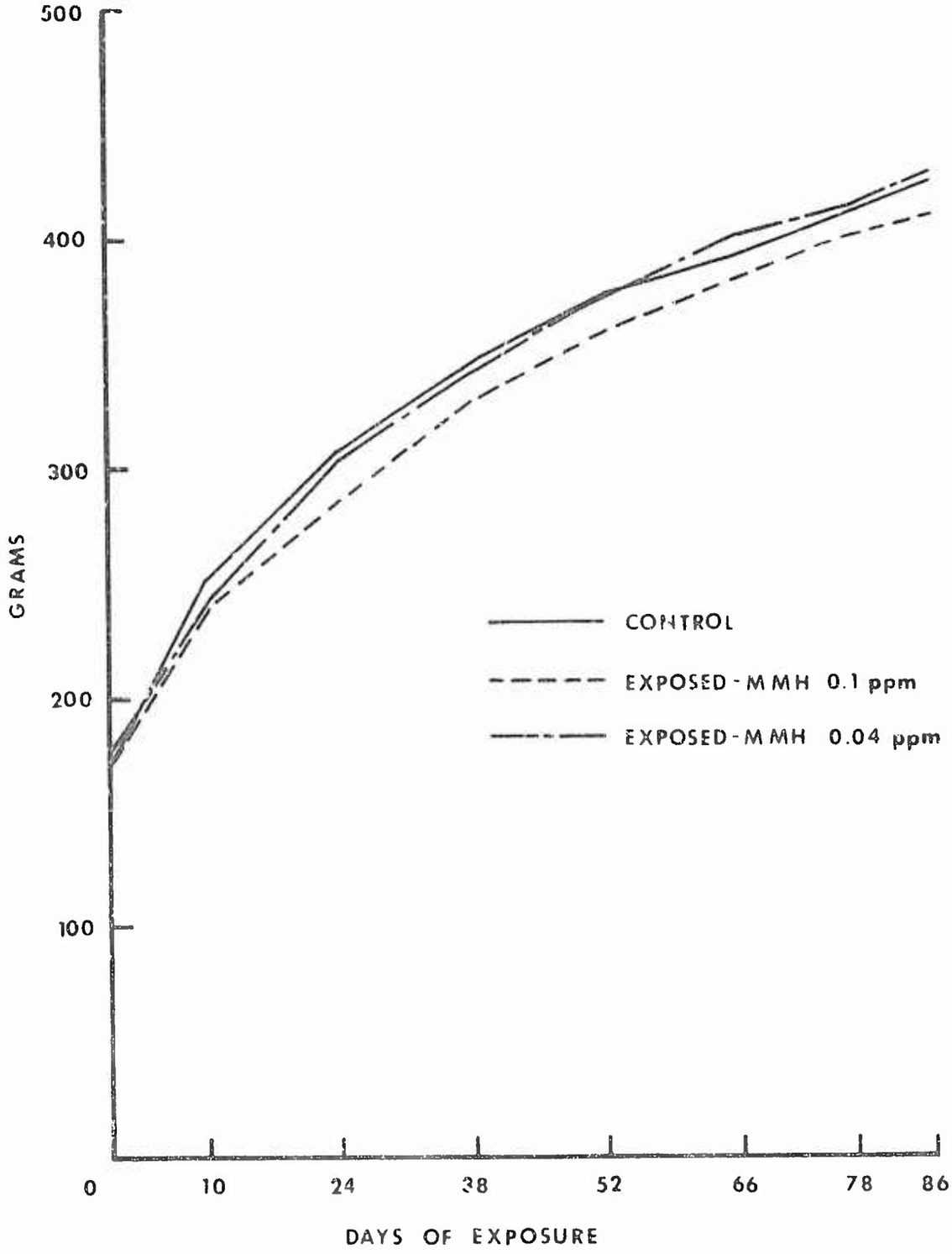


Figure 3. Growth rates of rats continuously exposed to MMH.

TABLE 2. EFFECT OF 90-DAY CONTINUOUS MMH EXPOSURE ON RAT ORGAN WEIGHT AND ORGAN TO BODY WEIGHT RATIOS

Organ	Unexposed Control	0.10 ppm Exposed	0.04 ppm Exposed
	Mean Organ Weights* (Grams)		
Heart	1.4	1.3	1.3
Lung	1.8	1.8	1.8
Liver	11.3	11.2	11.4
Spleen	0.8	0.8	0.8
Kidney	3.0	3.0	3.0
	Mean Organ to Body Weight Ratios* ($\times 10^{-2}$)		
Heart	0.338	0.335	0.319
Lung	0.432	0.456	0.437
Liver	2.763	2.823	2.755
Spleen	0.194	0.212	0.186
Kidney	0.735	0.747	0.718

*N = 20

TABLE 3. EFFECT OF CONTINUOUS MMH EXPOSURE TO RATS ON BLOOD MEASUREMENTS (MEAN VALUES)

	Control Group	Exposed Group 0.10 ppm	Exposed Group 0.04 ppm	
45 Days Exposure				
HCT (Vol %)	44	41**	42**	(1)
HGB (g %)	16.0	15.0**	15.6*	(1)
RBC (x 10^6)	8.2	7.5**	7.6**	(1)
WBC (x 10^3)	6.5	6.9	7.3	(1)
T. Phos. (mg %)	7.1	7.3	8.1	(2)
Alk. P'tase. (Int. Units)	154	146	142	(2)
90 Days Exposure				
HCT (Vol %)	44	43	44	(1)
HGB (g %)	16.7	15.4	15.7	(1)
RBC (x 10^6)	7.0	6.1*	7.6	(1)
WBC (x 10^3)	6.3	6.6	7.2	(1)
T. Phos. (mg %)	6.2	7.0*	6.7*	(2)
Alk. P'tase. (Int. Units)	117	117	117	(2)

* Significant at the 0.05 level

** Significant at the 0.01 level

(1) - N = 30

(2) - N = 10 pooled samples from 3 rats each.

TABLE 4. EFFECT OF CONTINUOUS MMH EXPOSURE ON SERUM TOTAL PHOSPHORUS LEVELS IN DOGS (MEAN mg %)

Exposure Weeks	Control Group	Exposure Group 0.10 ppm	Exposure Group 0.04 ppm
- 2	5.9	5.5	6.4
0	6.1	6.2	5.4
2	5.1	5.0	4.6
4	4.4	5.0	4.8
6	4.2	5.0*	4.7
8	4.4	5.1	4.6
10	4.2	5.2**	4.7
12	4.7	5.7*	5.1
13	4.0	4.9*	4.7

* Significant at the 0.05 level

** Significant at the 0.01 level

N = 8

TABLE 5. EFFECT OF CONTINUOUS MMH EXPOSURE ON SERUM ALKALINE PHOSPHATASE LEVELS IN DOGS (MEAN INTERNATIONAL UNITS)

Exposure Weeks	Control Group	Exposure Group 0.10 ppm	Exposure Group 0.04 ppm
- 2	89	103	85
0	131	180	144
2	101	211**	130
4	74	240**	101
6	67	354**	95
8	64	346**	99*
10	81	399**	118
12	63	302**	90
13	63	356**	85

* Significant at the 0.05 level

** Significant at the 0.01 level

N = 8

TABLE 6. EFFECT OF 90-DAY CONTINUOUS MMH EXPOSURE ON CLINICAL BLOOD MEASUREMENTS IN DOGS AND MONKEYS (MEAN VALUES)

	Unexposed Control	0.10 ppm Exposed	0.04 ppm Exposed
Dogs (N = 8)			
HCT (Vol %)	49	44**	47
HGB (g %)	18.1	15.1**	17.0
RBC (x 10^6)	6.26	4.73**	5.41
WBC (x 10^3)	13.6	15.1	11.9
Reticulocytes (%)	0.7	2.4	2.1
Monkeys (N = 4)			
HCT (Vol %)	40	38	38
HGB (g %)	13.8	13.8	12.7
RBC (x 10^6)	5.20	4.72	4.44
WBC (x 10^3)	8.2	7.2	6.8
Reticulocytes (%)	0.8	1.0	1.8

** Significant at the 0.01 level

One monkey in the 0.04 ppm exposure group died on the 10th day of exposure. At necropsy a preexisting condition of amyloidosis was observed. There was no evidence of any relationship of the MMH exposure to death and the monkey was excluded from the experimental group.

The hematologic effects of continuous exposure to 0.10 ppm MMH were consistent with the dose response previously reported at higher exposure levels (MacEwen and Haun, 1971). However, continuous MMH exposure at an atmospheric concentration of 0.04 ppm did not significantly alter the hematology of the test animals and had no effect on rat growth. On the basis of this experimental data we believe that 0.04 ppm monomethylhydrazine would be a safe threshold limit value for continuous exposure in confined working areas.

A Proposed 24-Hour Emergency Exposure Limit for Monomethylhydrazine (MMH)

Emergency exposure limits for MMH have been established for brief periods of time up to 60 minutes in duration. These limits, recommended by the Committee on Toxicology of the National Academy of Sciences-National Research Council, were based on animal experimentation and confirmed by exposures of human volunteers (MacEwen et al., 1969, 1970).

The principal acute toxic action of MMH is on the central nervous system (CNS), and is manifested by severe convulsions resulting in death (Jacobsen et al., 1955; Haun et al., 1970). Exposed animals may exhibit varying degrees of methemoglobin formation, slight in most species other than the dog, and a transient hemolytic anemia as well as appreciable kidney damage can occur in dogs, but these phenomena are considered to play a relatively unimportant role in the acute lethal action of MMH.

Signs of toxicity after administration of MMH are similar in all species, with the exception that vomiting occurs in dogs prior to the onset of convulsions. Convulsions in mice and rats are immediately fatal, but may be repeated several times before death occurs during a convulsive episode in dogs and monkeys. Convulsions in large animals do not necessarily lead to death.

A common finding in all species after lethal exposure to MMH is pulmonary congestion with some hemorrhage, hepatic congestion of varying degrees, and swelling of the renal tubular epithelium which is frequently glassy and eosinophilic in appearance. In dogs and monkeys whose brain tissues were examined, subarachnoid hemorrhage was frequently observed. This condition is probably related to the severe convulsions that preceded death, as is the consistent finding in dogs of remarkably bloodless spleens in which the sinusoids are practically empty. In some cases, the splenic smooth muscle bundles appear thickened and contracted.

In dogs and monkeys that survived near-lethal exposures to MMH and were sacrificed serially over a period of approximately 60 days after the exposure, visceral congestion was still apparent although not as severe as in those animals that died during the exposure. The most common and persistent finding was kidney damage which ranged from mild swelling of the tubular epithelium to vacuolization and coagulative necrosis of the epithelial cells. The renal changes observed in dogs were more extensive than those in monkeys. After exposure to subconvulsive doses of MMH, dogs exhibit hematuria, hemoglobinuria, methemoglobinuria and cast formation. As mentioned previously, histopathologic examination of the kidneys revealed proteinaceous precipitates in the proximal tubules and moderate to severe degeneration of the proximal tubules with necrosis in many cases.

Potential applications for future use of MMH in confined working areas suggested the need for Emergency Exposure Limit (EEL) values for periods up to 24 hours in duration. Consequently, animal experiments using the methodology and techniques reported by MacEwen (1969) were conducted.

Since dogs have been shown to be the most MMH sensitive species, preliminary experiments utilized these animals. Continuous 24-hour MMH exposures were provided to groups of two beagle dogs each at 1, 2, 5 and 10 ppm. These animals were maintained for 30 days postexposure for comparison with two exposed control dogs. Exposure at 2 ppm MMH produced

a 5-7% hemolysis of red blood cells through the methemoglobin-Heinz body transformation mechanism. This effect was followed by reticulocytosis which brought hemoglobin, REC and hematocrit values back to preexposure levels within three weeks. The same effects were seen to a greater degree after 24-hour continuous exposure to 5 and 10 ppm MMH.

No measurable decrease in the hematologic measurements was seen in the dogs exposed under the same conditions to 1 ppm MMH. Consequently, the 1 ppm MMH concentration was selected for a more comprehensive experiment, exposing 8 beagle dogs, 8 Rhesus monkeys and 50 albino rats (Sprague-Dawley strain) and observing them for comparison with unexposed controls for a 30-day period. The exposed and control rats were divided into two groups of 25 each. One group was killed in subgroups of two each three times weekly to obtain blood samples for hematology measurements while the others were held the full 30 days for growth measurements and pathologic evaluation at termination of the experiment.

No significant changes were seen in any of the measured parameters of hematology, serum alkaline phosphatase, total phosphorus, rat growth rate or red blood cell fragility. All three species tested (dogs, monkeys and rats) were not significantly different from preexposure values or from control animals of the same species during the 30-day postexposure observation period.

At the end of the 30-day postexposure period all animals were necropsied and gross pathologic examinations performed. Tissue samples were harvested and sent to others for evaluation. There were no gross pathologic changes related to the exposure in any of the experimental animals.

The results of these experiments would indicate that 1 ppm MMH is a safe concentration, and it is proposed as the tentative 24-hour Emergency Exposure Limit.

Continuous and Intermittent Inhalation Exposures of Animals to Low Concentrations of Hydrazine

Hydrazine (N_2H_4) is a highly reactive reducing agent which is widely used an an intermediate in organic synthesis and either singly or in combination with other hydrazines such as 1, 1 dimethylhydrazine or methylhydrazine as a missile propellant. Hydrazine is a colorless liquid with a molecular weight of 32.05, density of 1.008 g/ml and a vapor pressure of 14.4 mm Hg at 25 C.

Hydrazine is a strong convulsant at high doses but may cause central nervous system depression at lower doses. Animals may die acutely of convulsions, respiratory arrest, or cardiovascular collapse within a few hours of an acute exposure by any route of administration, or may die 2 to 4 days

later of liver and kidney toxicity (Weir et al., 1964; Witkin, 1956). Jacobson et al.,(1955) reported the four hour inhalation LC_{50} value as 252 ppm (330 mg/m^3) for the mouse and 570 ppm (750 mg/m^3) for the rat.

House (1964) exposed monkeys, rats and mice to a hydrazine concentration of 1.0 ppm continuously for 90 days. Though mortality was very high, some animals survived the experiment. Ninety-six percent of the rats and 98% of the mice died during the exposure while monkeys proved to be the most resistant species with only a 20% mortality.

Comstock et al. (1954) exposed dogs to 14 ppm N_2H_4 vapor on a 6-hour per day, 5 days per week basis. Two of four dogs died during the third and fifteenth weeks in a debilitated condition. The dog that died during the fifteenth week had a severe convulsive seizure prior to death. Prior to death, both dogs showed signs of anorexia and general fatigue. Changing diets and forced feedings resulted in the survival of the remaining two dogs.

The present Threshold Limit Value (TLV) published by the American Conference of Governmental Industrial Hygienists (1972) for N_2H_4 is 1 ppm or 1.3 mg/m^3.

To compare the effects of repeated 6 hour per day, 5 days per week (industrial type) exposures with continuous exposures of equivalent concentrations and to evaluate the safety factor of the current TLV, four concentration levels were selected for the 26 week exposure of four animal species. The concentrations selected were: 1.0 ppm and 0.2 ppm for continuous exposures and 5.0 ppm and 1.0 ppm for intermittent daily exposures. These concentrations would result in the following CT (concentration x time) values:

1.0 ppm continuous = 168 ppm-hours per week

5.0 ppm intermittent = 150 ppm-hours per week

1.0 ppm intermittent = 30 ppm-hours per week

0.2 ppm continuous = 33.6 ppm-hours per week.

Thus, the 1.0 ppm continuous and the 5.0 ppm intermittent studies would be relatively equivalent and the 1.0 ppm intermittent and 0.2 ppm continuous would also be comparable.

The four exposed groups and a control group consisted initially of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats and 40 female ICR mice.

Each group of animals was housed in a separate Thomas Dome at ambient pressure. The air flow, pressure, relative humidity and temperature were all controlled automatically. Air flow was maintained at 40 cfm, relative humidity at 50% $\pm$ 10% and temperature at 72 $\pm$ 5 F. The absolute pressure within the exposure chamber was slightly negative (725 mmHg) to insure a tight seal and prevent contamination of the surrounding laboratory air with N_2H_4 vapor.

Anhydrous hydrazine, 97% pure, was introduced through a stainless steel needle into a heated air stream by means of a single syringe feeder. Concentrations could be adjusted by either changing the speed of the syringe feeder or by adjusting the air flows through the domes or both. The concentration in each dome was monitored using an AutoAnalyzer as described for MMH analysis (Geiger, 1967). The two continuous domes and the control dome were sampled for 40-minute periods in succession. The intermittent domes were each sampled for 30 minutes of each hour. The control dome was sampled during each cycle for baseline values as the baseline changed slightly with the buildup of animal waste in the dome. During the daily routine maintenance of the domes all remaining animal food was replaced with fresh food to prevent, as much as possible, the ingestion of any hydrazine that may have been absorbed by the food pellets.

Bone marrow was examined from five rats per group removed after 8, 16 and 26 weeks exposure. Blood samples were taken for hematocrit, hemoglobin and RBC determinations by cardiac puncture, then the animals were sacrificed and submitted for gross and histopathology examinations. All rats were weighed on a biweekly schedule.

At the conclusion of the study ten rats were removed for lifetime retention and observation for possible carcinogenic effects. The rats that remained were sacrificed and submitted for gross and histopathological examination.

Ten mice from each group are also being held for lifetime observation. The remaining mice, used primarily for mortality information during exposure, were sacrificed and submitted for gross and histopathology examinations at the conclusion of the exposures. Mouse weights were not monitored.

At sacrifice, the brains were perfused in 4 of the 1 ppm continuously exposed dogs and 2 control dogs for signs of possible central nervous system effects. Bone marrow was sampled from 4 dogs of each exposure group. Organ weights were measured for heart, lung, liver, kidney and spleen for all species except mice. Because other investigators have reported fatty livers in animals exposed to hydrazine vapor, all tissues were treated with Oil Red O, fat specific, stain.

To determine whether recovery from any pathological effects took place following exposure, six dogs, two each, from the control group, the 1 ppm continuous exposure group and the 5 ppm intermittent group were kept for six weeks following the termination of exposures. Biweekly weighings, hematology, and chemistry tests were made on these dogs during the post-exposure period.

Examinations were made for Heinz bodies, and methemoglobin values were determined on all monkeys and four dogs per group prior to the start of the study, biweekly during the first month and monthly thereafter. Osmotic RBC fragility tests were performed on four dogs per group on the above schedule.

Hematology and blood chemistry determinations shown in Table 7 were made on dogs and monkeys prior to the start of the study and biweekly for the duration of the study.

TABLE 7. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON DOGS AND MONKEYS EXPOSED TO HYDRAZINE

Hematology	Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
Total RBC	Cholesterol
Total WBC	Calcium
Differential	Inorganic Phosphorus
Reticulocyte Count	Total Bilirubin
Mean Corpuscular Volume (MCV)*	Albumin/Globulin
Mean Corpuscular Hemoglobin (MCH)*	Total Protein
Mean Corpuscular Hemoglobin Concentration (MCHC)*	BUN
	Glucose
	Alkaline Phosphatase
	SGOT
	SGPT
	Creatinine
	Chloride
	Triglycerides

*Dogs only

Blood indices, the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated for dogs only using their biweekly hematocrit, hemoglobin and red blood cell values.

An early sign of toxic stress was some minimal eye irritation noted in the monkeys exposed to the two highest exposure levels. The monkeys exposed to 5 ppm intermittently showed eye irritation sporadically during the remainder of the study but at no time was it regarded as severe. The fur of the mice exposed to the two highest concentrations, at times, appeared yellow and rough but this, again, was not a continuous condition.

There were no monkey deaths and only one rat death during the duration of the study. The rat death, which occurred early in the study at the lowest concentration level, was not attributed to hydrazine exposure. The majority of mouse deaths occurred during the first month of exposure and exhibit a direct relationship with N_2H_4 dose received as shown in Table 8. This could also be indicative of an induced tolerance to the contaminant (possibly liver enzyme changes) after the first four weeks resulting in a decrease in the mortality rate.

Two dogs died after 16 weeks of continuous exposure to 1 ppm N_2H_4 vapor. Prior to death these dogs showed a suppression of appetite and refused to eat. They spent most of their time lying down in their cage showing no interest in the activity taking place around them. They, along with several other dogs from the two highest concentrations, showed severe weight losses. Although food consumption was not measured, it was noticeably reduced. As shown in Figure 4, a maximum loss of about 3/4 of a kilogram is seen after four weeks of intermittent exposure to 5 ppm, after which they apparently recover. However, the weight loss among these dogs begins showing again after 16 weeks and continues to the termination of the exposures. A 2-kilogram mean weight loss occurred at six weeks for dogs exposed to 1 ppm interrupted by a sharp recovery at eight weeks and then followed by a steady loss to 16 weeks. The weight depression seen between 8 and 16 weeks was due, in part, to the influence of the severe weight losses of two dogs which died shortly after the 16-week weighing. A steady recovery is seen following 16 weeks; however, the mean body weight of this dog group was still below control values at the termination of exposure.

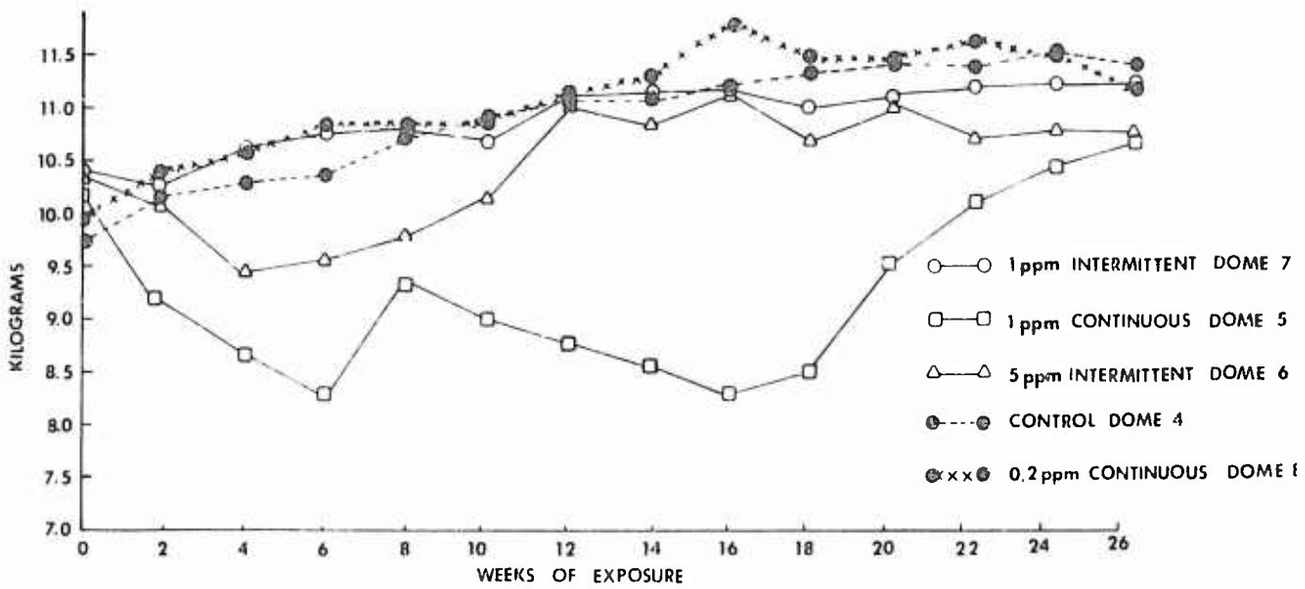


Figure 4. Mean weight change in dogs exposed to hydrazine.

TABLE 8. EFFECT OF HYDRAZINE EXPOSURE ON MOUSE MORTALITY (N = 40/GROUP)

Hydrazine Exposure Condition	% Total Mortality
1.0 ppm, Continuous	55
5.0 ppm, Intermittent	35
1.0 ppm, Intermittent	7.5
0.2 ppm, Continuous	2.5
Control	0

The mean body weights of all groups of exposed monkeys did not differ significantly from the controls at any time in the study (Figure 5). The rats show a dose related depression of growth with the two high concentrations being affected most. Figure 6 shows that all rat groups continued to gain in weight throughout the study but the rate of weight gain in the test animals was suppressed when compared to the controls. The differences were statistically significant for all exposed rat groups except the 0.2 ppm exposure group study which showed differences from controls between weeks 2 and 10 only.

The dog and monkey methemoglobin values determined during the course of the study, when compared with the control values, showed no differences. There was no significant buildup of Heinz bodies found at any time during the scheduled examinations. Blood indices, calculated for all dogs, showed no statistical differences when values for exposed groups were compared with those of the control group. Cell populations were normochromic and normocytic.

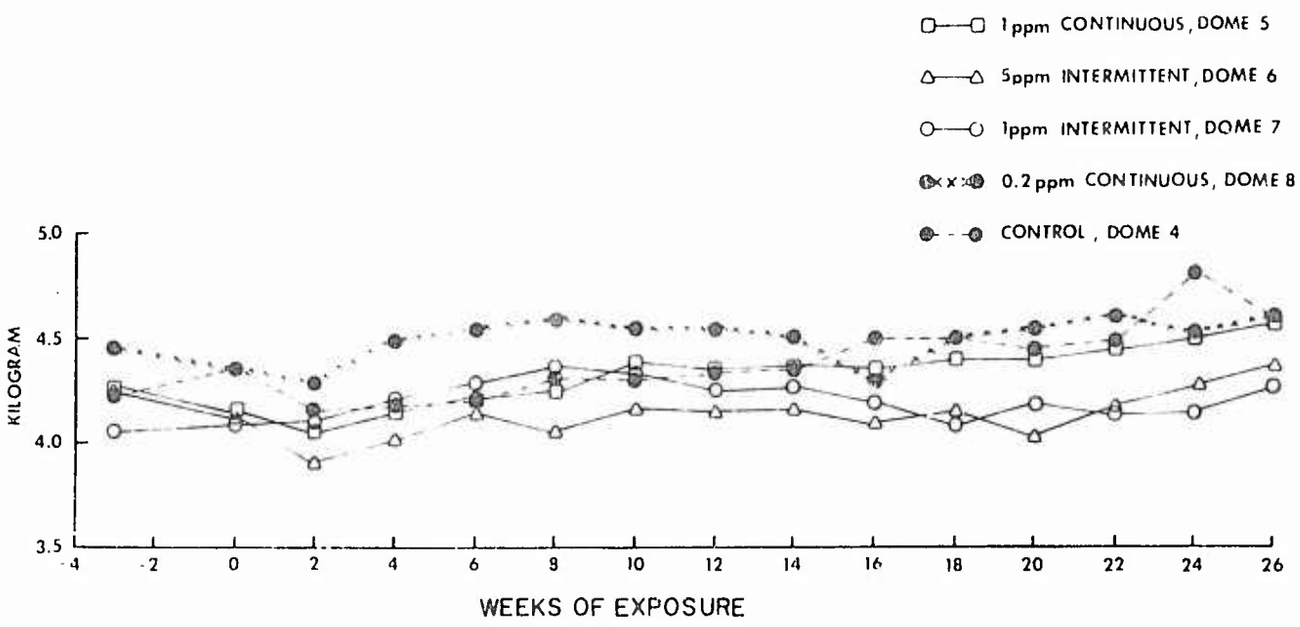


Figure 5. Effect of a 6-month hydrazine exposure on monkeys.

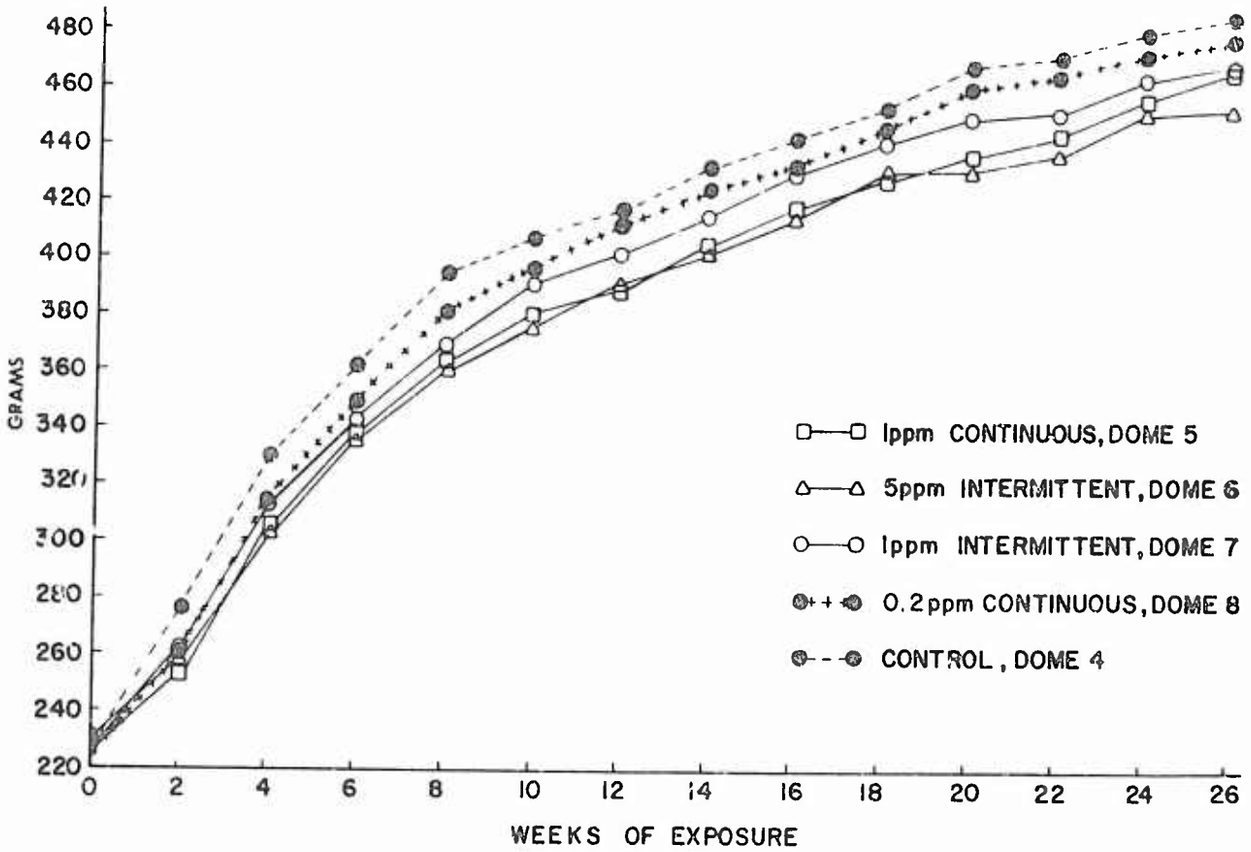


Figure 6. Effect of a 6-month hydrazine exposure on rats.

The dog erythrocytes sampled after two and four weeks exposure, tested for osmotic fragility, showed no differences from the controls. At eight weeks there was some increased resistance to osmotic hemolysis in cells from dogs exposed to the two higher doses, but too small to be considered significant. However, at 12 weeks and for the remainder of the study, the effect was larger and dose dependent. Figure 7 shows the osmotic fragility of red blood cells sampled from dogs continuously exposed to 1.0 ppm hydrazine for 16 weeks compared with controls. This figure is typical of the resistance to osmotic pressure found in each measurement throughout the remainder of the study. There was a definite dose related response of decreased red blood cell fragility resulting from N_2H_4 exposure. This response was not seen in monkeys.

Figure 8 is a plot of dog hematocrit values for the 26 weeks of the experiment. The patterns of effect on hemoglobin and red blood cell count (Figures 9 and 10) closely parallel that of the hematocrit. Only the 1.0 ppm continuous and 5 ppm intermittent exposures had any effect on hematological parameters in dogs. Dogs exposed to these concentrations experienced significant decreases in HCT, RBC and HGB after 2 months which remained depressed for the rest of the study. Reticulocyte counts were unaffected by hydrazine exposures. Hemoglobin, RBC and HCT values returned to control levels within 2 weeks postexposure in the 2 dogs from each experimental group held for further observation.

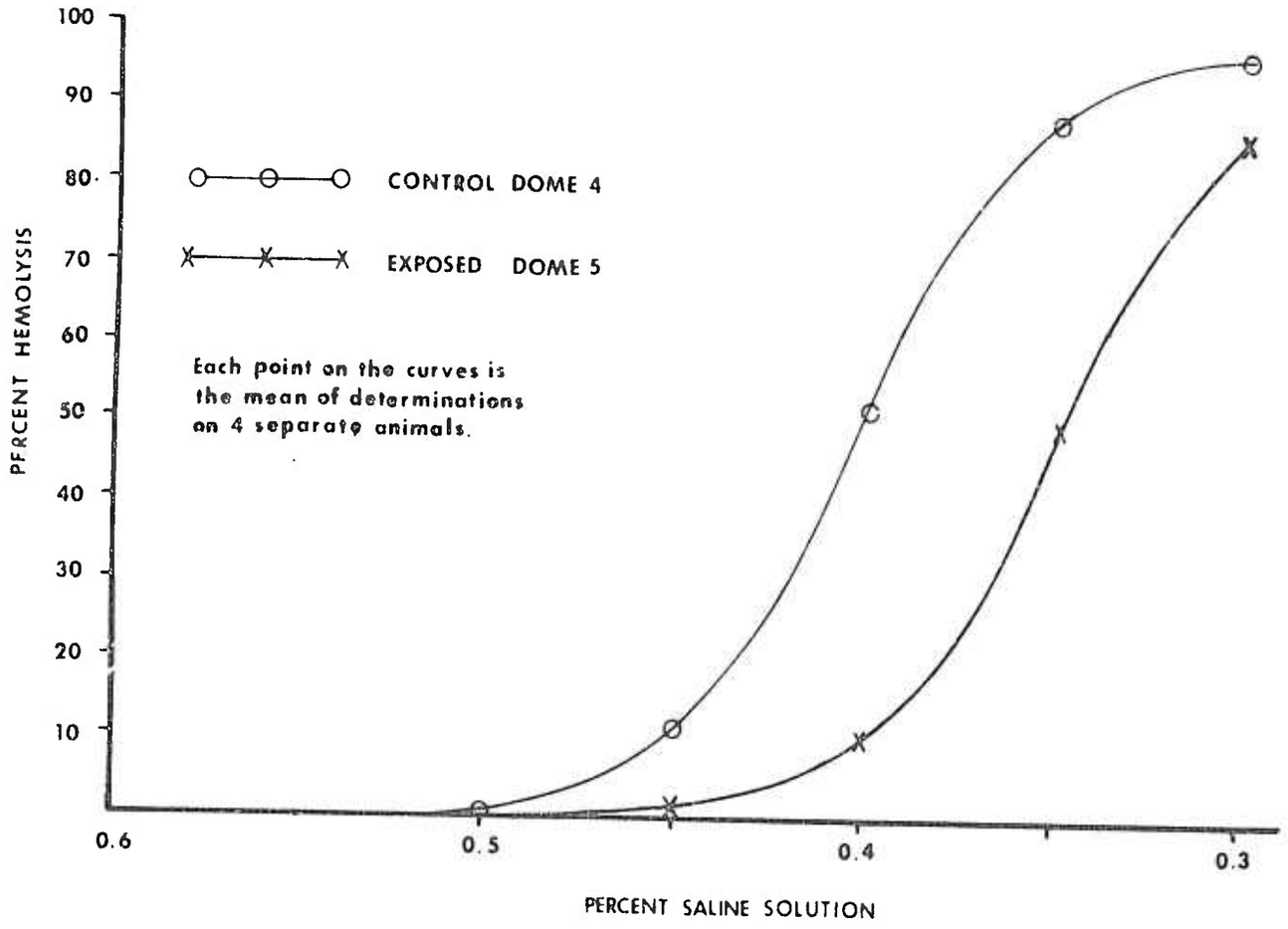


Figure 7. Osmotic fragility of red blood cells from dogs continuously to 1 ppm hydrazine over a 16-week period.

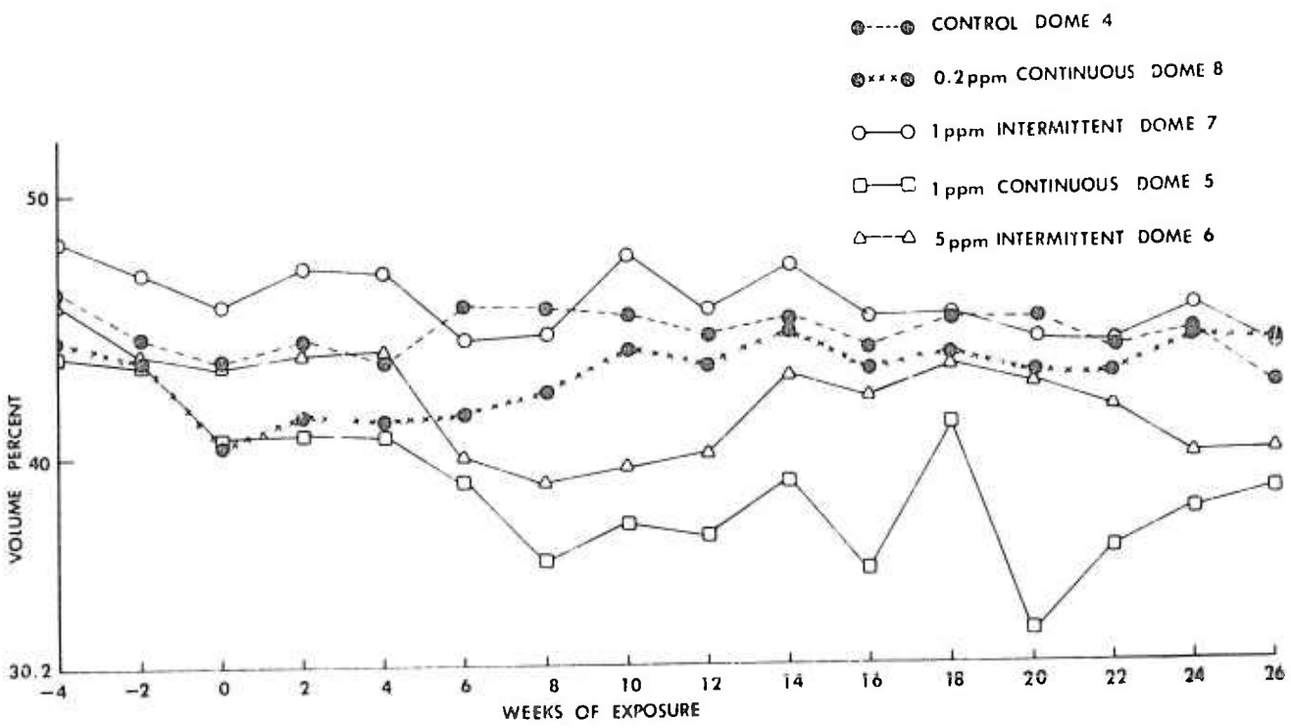


Figure 8. Effect of hydrazine exposure on hematocrit values in dogs.

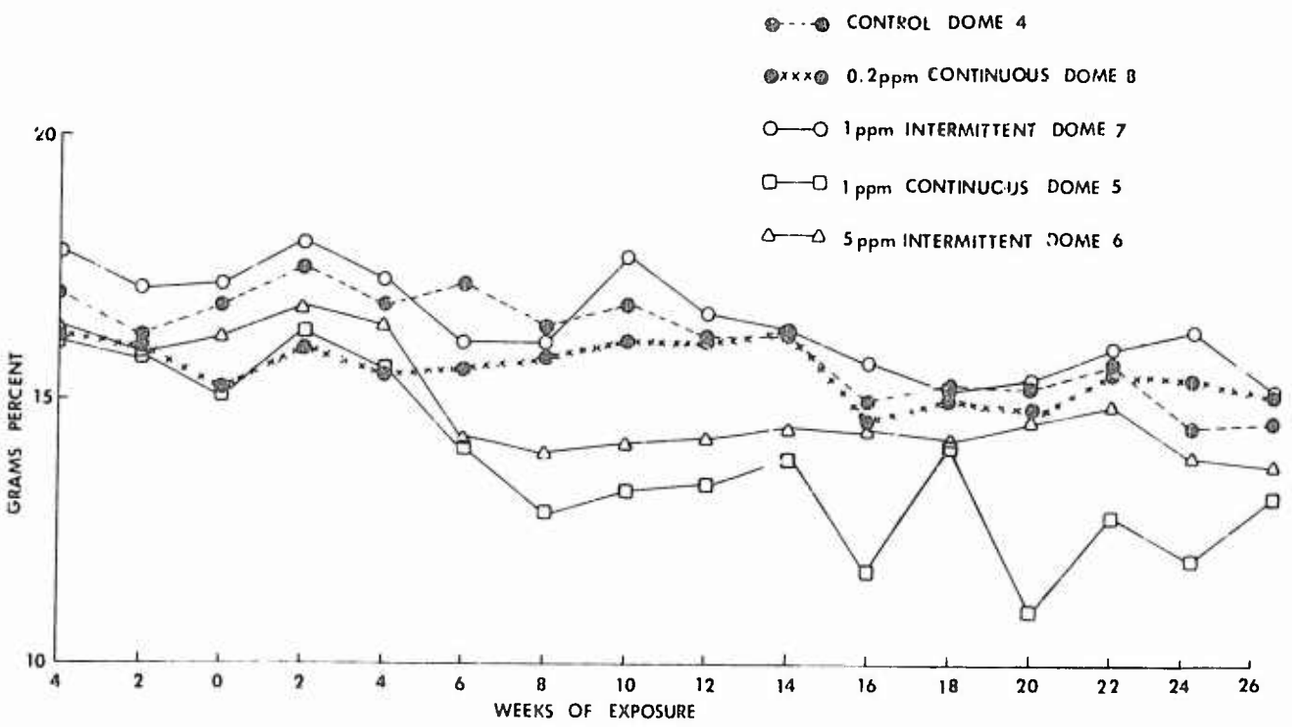


Figure 9. Effect of hydrazine exposure on hemoglobin levels in dogs.

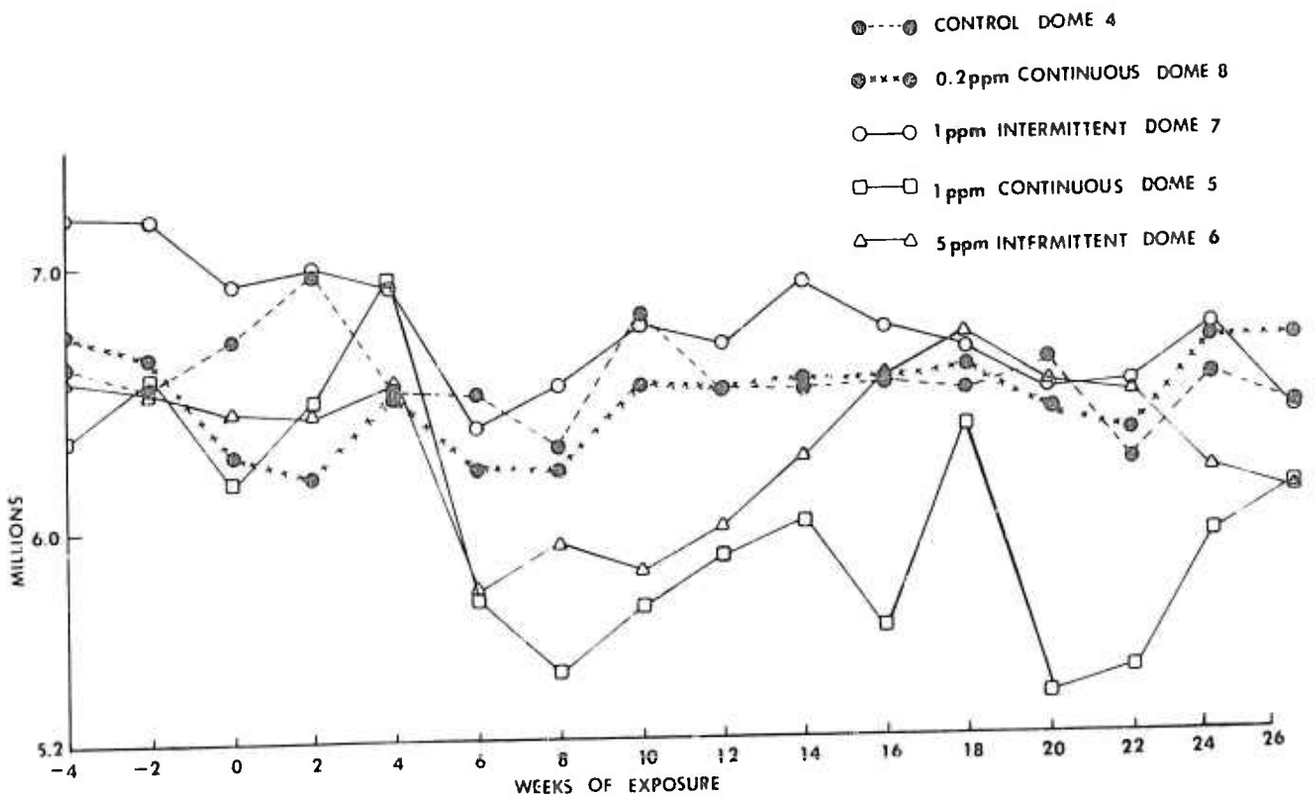


Figure 10. Effect of hydrazine exposure on red blood cell counts in dogs.

One dog experienced mild tonic convulsions after 3 months of continuous exposure to 1.0 ppm N_2H_4 and had 2 additional convulsive episodes on the same day during the 5th exposure month. Each seizure lasted approximately 30 minutes. The dog appeared normal at other times and survived the six month exposure period.

At no time during the study were there any biologically significant differences between control and exposed rats and monkeys with respect to hematology measurements. Rat bone marrow examination showed normal ratios of myeloid to erythroid elements.

Examination of the clinical chemistry data consisting of 13 separate determinations performed on a regular biweekly schedule for dogs and monkeys showed no significant differences between exposed and control groups.

Organ weights of N_2H_4 exposed rats were unchanged from control animals; however, the depressed growth rates resulted in increased organ to body weight ratios to which no biological significance can be attributed.

Gross pathology examinations of the mice that died during exposure failed to reveal anything that could be defined as treatment-related. Gross examination of the two dogs that died after 16 weeks of continuous exposure to 1.0 ppm vapor showed a general emaciation with no subcutaneous fat present, atrophy of the muscles, spleens approximately 1/2 normal size and fatty livers. Most of these signs are indicative of starvation.

No treatment-related gross pathology was found in any species when examined at the conclusion of the study. Micropathological examination of the tissues is now in progress.

Certain conclusions can be drawn at this time. Chronic exposure to the two high level hydrazine concentrations (1 ppm continuous and 5 ppm intermittent) causes mortality in mice. Continuous exposure to 1 ppm hydrazine vapors is also fatal to dogs, resulting in a 25% mortality.

Blood cell destruction occurred in the dogs exposed to 1.0 ppm N_2H_4 continuously and 5 ppm intermittently. This was demonstrated by the reduced number of red blood cells and the decreases in hematocrit and hemoglobin values. Thienes et al., (1948) found red cell destruction when administering hydrazine by stomach tube. In chronic studies, rats receiving doses equivalent to only 1/20 of the LD_{50} had decreases in red cells of as much as 50%. We found decreases in red cells up to 13% in the 1.0 ppm continuous dog group.

Anorexia and body weight effects were noted in rats and dogs exposed to the two highest concentrations of hydrazine vapor. Several dogs became weak and thin and lost appetites. The rats, although not showing an obvious appetite loss, did have subnormal weight gains.

Continuous exposure may result in central nervous system effects, such as the convulsions demonstrated on three separate occasions by one dog exposed continuously to 1 ppm N_2H_4.

The indices of blood destruction, i.e., decreases in RBC, hematocrit and hemoglobin values, were back to normal after two weeks when examined in the dogs retained at the conclusion of the study. This indicates that the effects are reversible and that the blood parameters will return to normal if the contaminant exposure is stopped.

Histopathologic examinations have been completed for monkeys, dogs and mice except for brain tissues. Tissues from rats have not yet been evaluated nor have bone specimens from terminal sampling.

The only apparent histologic difference between exposed dogs, monkeys and mice and their controls in the tissues examined is a slight to moderate increase of fat accumulated in livers of mice and monkeys at all exposure levels. Dogs exhibit this effect only at the 2 highest doses (1.0 ppm N_2H_4 continuous and 5.0 ppm intermittent exposures). Final evaluation of these experiments awaits completion of examinations of rat tissues, dog and monkey brain tissue and the bone marrow specimens.

Percutaneous and Oral Administration Studies for Classification of Toxicity Ratings of Transportable Chemical Agents

In last year's annual report the toxicity of several compounds of interest to the Department of Transportation (DOT) was discussed. This was done in the light of a reexamination of existing data or the determination of data on those compounds where no previous data existed. The information so gathered was used to help reclassify these compounds into categories which would set shipping and handling requirements concomitant with the hazard associated with each compound. The results of these studies are published in a separate document (Back et al., 1972).

During the current reporting period, seven new compounds were examined in this same light to provide additional information to the DOT. These compounds are listed below:

1. mixed cresols
2. allyl isothiocyanate
3. methyl isothiocyanate
4. methyl isocyanate
5. ortho-nitroaniline
6. ethyl chloroacetate
7. phenyl isocyanate

All seven compounds were administered to rabbits to determine skin absorption LD_{50} values, and three of the seven compounds (allyl isothiocyanate, methyl isothiocyanate and methyl isocyanate) were administered orally to both rats and mice to determine the LD_{50}'s in each of these species. These studies were designed to define the acute toxic response of the subject compound resulting from single accidental exposure and do not define the potential total hazard of carcinogenic risk or inhibition of performance of emergency duties including self rescue.

For the determination of oral toxicity, the following methods were used:

Male CFE rats (200-300 grams) and male CF-1 mice (20-30 grams) were used in this study.

All compounds were given as a suspension in corn oil. All suspensions were kept in a turbulent state while in use. Glass syringes with special oral dosing needles were used to administer the compounds to the rodents. The experimental animals were fasted overnight prior to administration of the oral dose. This allowed for more uniform absorption, since the amount of food in the stomach varies greatly from animal to animal in the unfasted condition. The injected volumes of test compounds for the rodents were approximately 0.01 ml/gm. This resulted in the average mouse receiving a volume of 0.25 ml, and the average rat a volume of 2.5 ml. Both mice and rats were weighed individually at the time of dosing to determine the proper dose volume.

Rangefinding doses were given for each compound. These consisted of intubating five rats and five mice each at three dose levels estimated from available evidence in the literature, or if information was not available, dose levels of 5 mg/kg, 50 mg/kg and 500 mg/kg were used. Results of the range-finding tests served to determine whether doses needed to be higher or lower. After finding the proper range, geometrically spaced doses were administered to determine the actual LD_{50}. Five rats and 5 mice were dosed at each level, and the LD_{50} with its 95% confidence limits was calculated using the moving average interpolation method of Weil (1952).

Deaths which occurred during the 14 days immediately following the administration of the single dose were included in the final mortality tally. Any animals which survived the 14-day postexposure period were sacrificed at that time.

For the dermal absorption toxicity determinations, female albino New Zealand rabbits (Pel-Freeze Bio-Animals, Inc.) weighing approximately 5 pounds were used as the experimental animals. All rabbits were clipped as closely as possible with an Oster clipper having surgical blades and a vacuum attachment. The back of the rabbits and the sides down to about half way to the stomach area were clipped from the saddle area of the shoulders to the top of the rear leg area.

The animals were individually weighed to determine the proper dose volume. The measured volume of the liquid material was then applied undiluted to the back of the rabbit and was divided as equally as possible between the two sides of the back. If the volume was sufficiently great, the dose was kept in place by applying 8-ply gauze patches over the liquid on each side of the back. A patch of latex rubber dental dam or vinyl plastic, whichever was most compatible with the compound being tested, was then applied over the entire back area where clipped and elastoplast tape was used to wrap the entire midsection of the rabbit to keep the gauze in place. Specially designed rabbit restraining harnesses (Newmann, 1963) were fitted to each rabbit at the time of treatment. These harnesses restricted undesirable movement of the rabbits, i.e., prevented them from chewing on the taped area. The harnesses did, however, allow the rabbits complete freedom to eat and drink during the 24-hour restraining period.

All dosing procedures were carried out in a fume hood due to the volatile and dangerous nature of the compounds being tested. Rubber protective gloves were worn at all times by the personnel involved in the dosing procedures. The rabbits were housed in individual cages kept in the hood during the dosing period.

All compounds were applied undiluted. The single solid material (ortho-nitroaniline) was applied in coarse powder form held in place with gauze patches and a rubber dental dam.

All test compounds remained in contact with the rabbit's skin for 24 hours. After this period of time, the gauze tape and harness were removed. The rabbits were observed for signs of toxicity or death during the 14 days immediately following dosing.

The results of the oral toxicity determinations are shown in Tables 9 and 10.

TABLE 9. ORAL TOXICITY OF VARIOUS COMPOUNDS TO RATS

Compound	Data Used to Calculate LD_{50} in mg/kg (mortality response, N=5)	LD_{50} (95% C.L.) in mg/kg
Allyl Isothiocyanate	150 (0), 300 (1), 600 (3), 1200 (5)	488 (235-1010)
Methyl Isothiocyanate	100 (0), 200 (2), 400 (5), 800 (5)	218 (108-443)
Methyl Isocyanate	37.5 (1), 75 (1), 150 (2), 300 (5)	138 (55-343)

TABLE 10. ORAL TOXICITY OF VARIOUS COMPOUNDS TO MICE

Compound	Data used to Calculate LD_{50} in mg/kg (mortality response, N=5)	LC_{50} (95% C.L.) in mg/kg
Allyl Isothiocyanate	125 (0), 250 (1), 500 (5), 1000 (5)	308 (194-688)
Methyl Isothiocyanate	37.5 (0), 75 (0), 150 (5), 300 (5)	106 (38-300)
Methyl Isocyanate	50 (0), 100 (1), 200 (5), 400 (5)	123 (78-195)

TABLE 11. DERMAL TOXICITY OF SEVEN COMPOUNDS IN RABBITS (24 Hour Exposure)

Compound	Data used to Calculate LD_{50} in mg/kg (mortality response, N=3)	LD_{50} (95% C.L.) in mg/kg
Mixed Cresols	1000 (0), 2000 (2), 4000 (3)	1782 (959-3386)
Allyl Isothiocyanate	62.5 (0), 125 (3), 250 (3)	88 (no C. L.)
Methyl Isothiocyanate	18.75 (0), 37.5 (2), 75 (3)	33 (18-64)
Methyl Isocyanate	800 (0), 1600 (1), 3200 (3)	1796 (945-3411)
Phenyl Isocyanate	4000 (0), 8000 (2), 16000 (3)	7127 (3744-13535)
Ortho-Nitroaniline	5000 (0), 20000 (0)	Below toxic
Ethyl Chloroacetate	100 (0), 200 (1), 400 (3)	225 (118-426)

The only solid material in the group of compounds tested , ortho-nitroaniline, was found to be below toxic levels for 24 hour skin absorption in rabbits. The highest dose shown in Table 11 for this compound was not fully absorbed and increasing the dose beyond this level would be uninformative.

The highest dose of phenyl isocyanate was of such a great volume (approximately 50-60 ml) that the entire trunk area was shaved and vinyl plastic, with gauze sponge inside, was wrapped around the entire midsection of the rabbit. This vinyl was then secured in place with elastoplast tape, and the injection of the material was then made directly under the collar so as to contain the entire volume.

There were few delayed deaths in rabbits given doses of any of the compounds under investigation. In general, they died during the initial 24-hour observation period or within two days postexposure (only four rabbits died beyond this time out of 60 used for the entire study). Several rabbits had severe burning or irritation of the skin following administration of these compounds, most notably the isothiocyanates and the isocyanates. In some cases, at the end of the 14-day observation period, the skin was necrotic and hair had not grown back in the places where the compound had been in contact with the skin.

Two of the seven compounds tested on rabbits for skin absorption toxicity were tested for oral toxicity in rats and mice in the previous DOT study (MacEwen and Vernot, 1972). These were the mixed cresols which had an LD_{50} of 1454 mg/kg and in mice of 561 mg/kg and ortho-nitroaniline which had an LD_{50} in rats of 3564 mg/kg and in mice of 1288 mg/kg. In the present study, the mixed cresols were seen to have a 24-hour skin absorption LD_{50} of 1782 mg/kg in rabbits. This indicates that the toxicity in rabbits for this compound is comparable to that in rats, and that the compound is equally well absorbed through the skin of rabbits or the G. I. tract of rats. Mice, however, had a considerably greater sensitivity to the mixed cresols and, therefore, had a much lower oral LD_{50} value than rats.

The ortho-nitroaniline was seen to have a 24-hour skin absorption LD_{50} greater than 20,000 mg/kg. In fact, no rabbits were killed at that dose, and the quantity of material was in such excess of that which could be absorbed that it is highly unlikely that this material would represent any appreciable toxicity danger by this route of administration. The ortho-nitroaniline was, however, readily absorbed from the G. I. tract of rats and mice, with the rats again being able to tolerate more than twice the dose tolerable to mice.

In comparing the three compounds tested for both oral toxicity in rats and mice and skin absorption toxicity in rabbits in the present study (allyl isothiocyanate, methyl isothiocyanate and methyl isocyanate), it was found that two of the compounds, the isothiocyanates, were much better absorbed through the skin than from the G. I. tract, while the methyl isocyanate was poorly absorbed through skin, but readily from the G. I. tract. The rat LD_{50} values were higher than mouse LD_{50} values in all three cases, with the methyl isothiocyanate LD_{50} in rats being about twice that in mice, the allyl isothiocyanate LD_{50} being slightly less than twice the mouse value, and the methyl isocyanate being approximately equal. The ready absorption of both isothiocyanates through rabbit skin was evident from the very low LD_{50} values obtained (88 mg/kg for the allyl, and 33 mg/kg for the methyl derivative In view of these findings extreme caution should be exercised when handling either of these isothiocyanates. An added factor is that these compounds were seen to react with latex rubber and hence use of gloves made of that material would not be an acceptable safety precaution. These materials did not, however, react with vinyl plastic.

Methyl isocyanate was poorly absorbed through skin by comparison to both isothiocyanates and was absorbed equally well from rat and mouse G. I. tracts. Hence methyl isocyanate can be considered less of a hazard from handling than from ingestion in contrast to the isothiocyanates.

Although classification of irritation effects of those compounds was not a part of this study, the very potent lacrimation induction by several of the compounds, most notably the isocyanates, was impossible to overlook. These properties would certainly have to be considered when establishing safety regulations for the handling and storage of these compounds.

Short-Term Animal Exposure to Carbon Monoxide (CO) and Hydrogen Cyanide (HCN) Singly and in Combination

A number of aircraft crashes and fires have resulted in passenger deaths which could not be attributed to traumatic injuries. Pathologic examinations and blood tests for cyanide and carboxyhemoglobin levels have also failed to establish a clear-cut cause of death other than smoke inhalation or severe burns. The cause of death in such cases may very likely be the result of inhalation of dangerous pyro-decomposition products present in the atmosphere of the burning aircraft cabin. Such exposures would be brief in nature since passengers would either escape or suffer fatal burns within a few minutes of the outbreak of the fire. Since it is impractical to monitor atmospheric concentrations of the pyro-decomposition products during actual aircraft fires,

only the blood levels of the compounds in the victims can be examined and an attempt made to correlate these with the conditions necessary to produce them.

Individually measured blood cyanide and carboxyhemoglobin values, although below those levels considered lethal, may be indicative of an additive or synergistic toxic response. There were, however, no published experimental data available to confirm this possibility. Acute toxicity tests on inhaled hydrogen cyanide for 5-minute exposures conducted in combination with carbon monoxide did not show any increased toxic response at carboxyhemoglobin levels of 25% saturation.

To determine whether there was a toxic interaction of some of the principal combustion products of aircraft fires, studies were conducted to (1) define blood levels of HCN and CO necessary to cause death in laboratory animals under controlled conditions, (2) determine the rate of uptake of each into the blood and (3) to determine whether exposure to both compounds simultaneously represented a greater hazard than each separately. From this data, it was hoped to define blood levels of each compound, and combinations of blood levels at which death or survival would be possible, above which survival would be impossible and below which survival would be assured.

The experimental animals used in these studies were 200-300 gram male Sprague-Dawley rats. Each group of animals was subjected to quality control examinations prior to use and appeared to be in good health.

All exposures were made in a modified Rochester Chamber (Leach et al., 1959; Haun et al., 1969) under dynamic airflow conditions. The experimental set-up is shown in Figure 11. For combined exposures an appropriate amount of carbon monoxide was passed through a flowmeter and mixed with metered amounts of hydrogen cyanide just prior to introduction into the chamber airflow. The hydrogen cyanide was supplied in liquid form in a cylinder. This cylinder was pressurized to about 40 pounds with nitrogen to permit uniform flow of small volumes of liquid HCN through a flowmeter. The HCN was then vaporized in a heated glass evaporator using a flow of 5 cfm predried air. The total chamber airflow was maintained at 50 cfm, 45 cfm from the preconditioning system, and 5 cfm from the contaminant generation system. A slight negative pressure was maintained in the chamber at all times to prevent leakage of the contaminants into the laboratory air.

Chamber concentrations of both HCN and CO were monitored continuously during all exposures. The HCN was monitored with a cyanide specific electrode after collection in 0.1 N NaOH absorber solution. Carbon monoxide was monitored with a nondispersive infrared analyzer, and concentration recorded on a strip chart recorder. It was determined experimentally that neither contaminant interfered with the analysis of the other for the exposure concentration range tested.

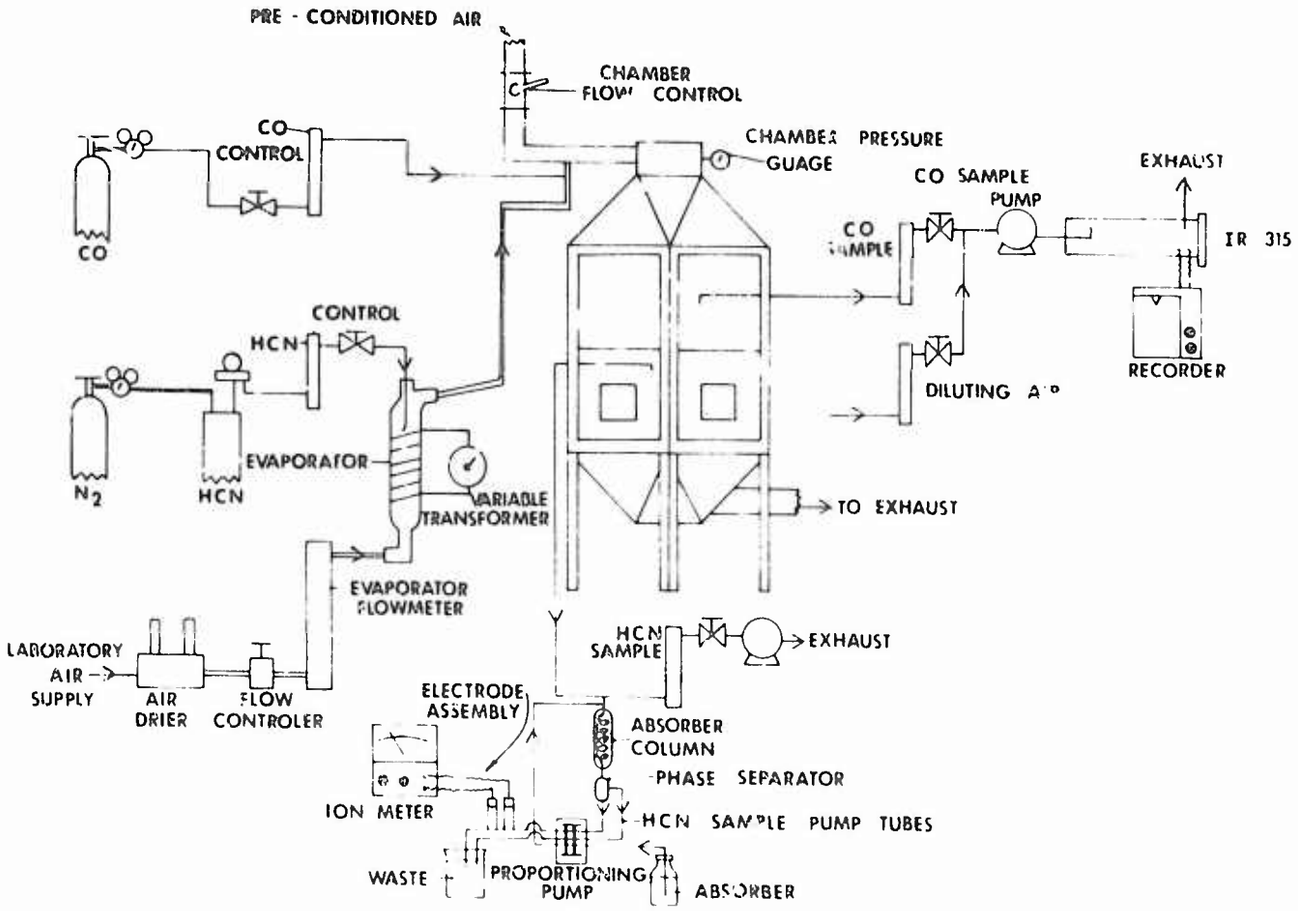


Figure 11. Rochester chamber system for HCN and CO combination exposures.

The desired concentrations of each contaminant were established in the chamber and then the rats were inserted into the chamber using the sliding cage drawers shown in Figure 12 (DiPasquale and Davis, 1971). All exposures were timed with a stopwatch. Each exposure had 10 rats, 5 per sliding cage. Individual rats were observed for toxic signs and mortality during exposure, and time to death was recorded.

Immediately upon completion of exposure, the rats were removed from the cages and bled with heparinized syringes by cardiac puncture. Bleeding the 10 animals required no longer than 2 to 2.5 minutes. It was determined that no significant change in carboxyhemoglobin occurred for this length of time postexposure.

Blood cyanide concentrations were determined by placing 1 ml of heparinized blood into the outer ring of a Conway diffusion dish. One ml of 0.1 N NaOH was then placed in the central ring and 0.5 ml of 10% sulfuric acid was added to the blood. The top was sealed immediately after addition to the acid, to keep the liberated cyanide in the diffusion dish. These dishes were then mixed and allowed to sit for 3.5 hours. After this waiting period, which was found to be optimal for maximum recovery of HCN, the lids were removed and the NaOH in the central ring was transferred with a Pasteur pipette into the cyanide ion electrode cell for analysis.

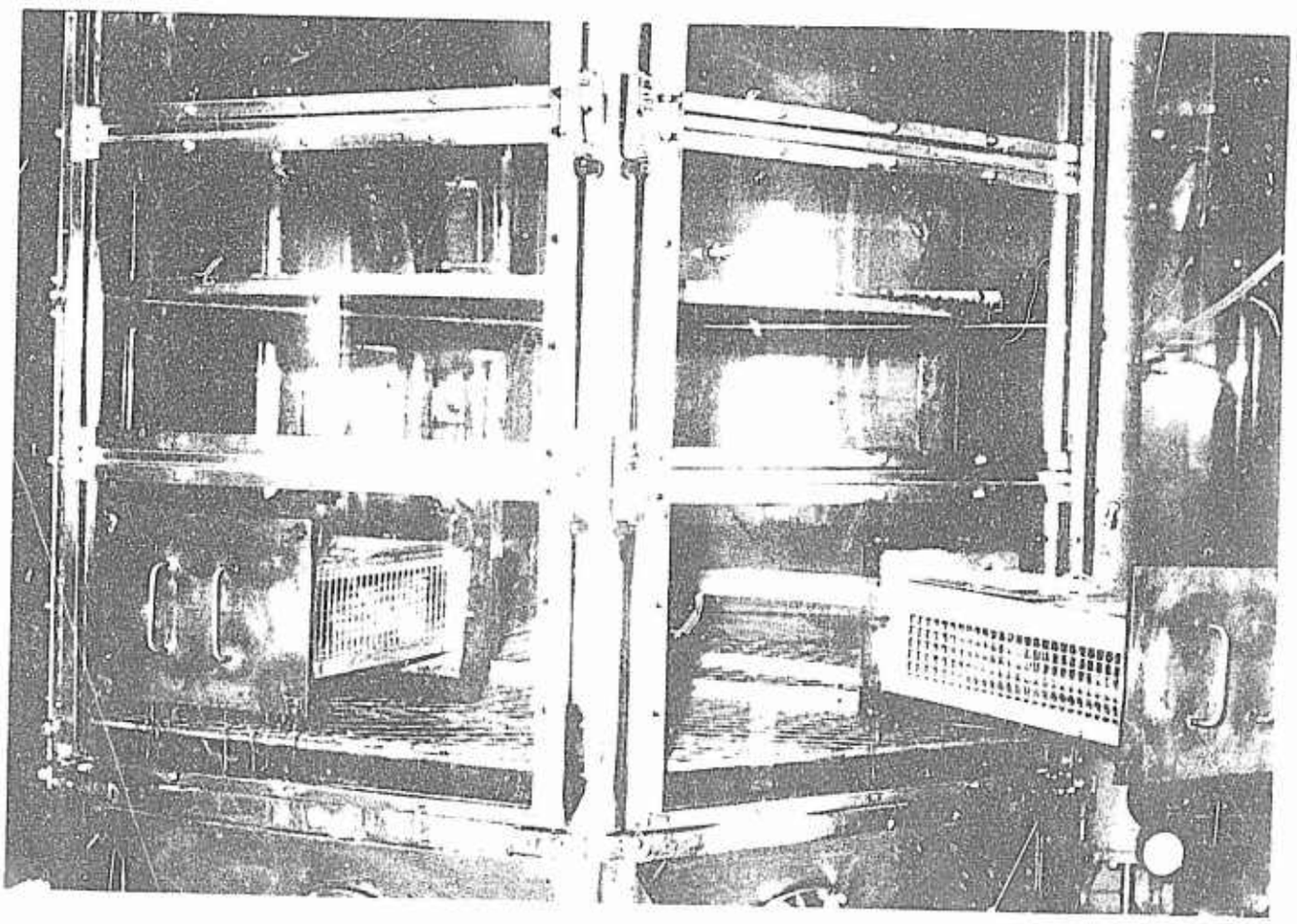


Figure 12. Rochester chamber modified to accept sliding cage drawers.

The amount of CO in blood was determined immediately following exposure by measuring carboxyhemoglobin content using a CO-Oximeter standardized for rat blood.

The first step in carrying out this study was the determination of LC_{50} values for HCN and CO individually. The LC_{50} values were determined for 5-minute exposures since this was thought to be a maximum exposure period for a victim of an airplane fire. All subsequent concentrations used in this study were based on the results of the 5-minute mortality curve. Figure 13 shows the mortality data for rats exposed to HCN. The important concentrations used in the balance of the study are marked on the curve. Figure 14 shows the mortality data for rats exposed to CO. Again, the important concentrations are marked on the curve.

To determine the rates of uptake of HCN and CO into rat blood, various concentrations of each were selected, and groups of 10 rats were exposed to these concentrations for varying lengths of time. The groups of rats were exposed to the 5-minute HCN LC_{50} for varying time periods less than 5 minutes and to the theoretical LC_{25}, LC_{10}, LC_2 and LC_0 for periods ranging from 3 to 10 minutes. For the determination of CO uptake, groups of rats were exposed to similar combinations of the LC_{50} and the theoretical LC concentrations for various time periods.

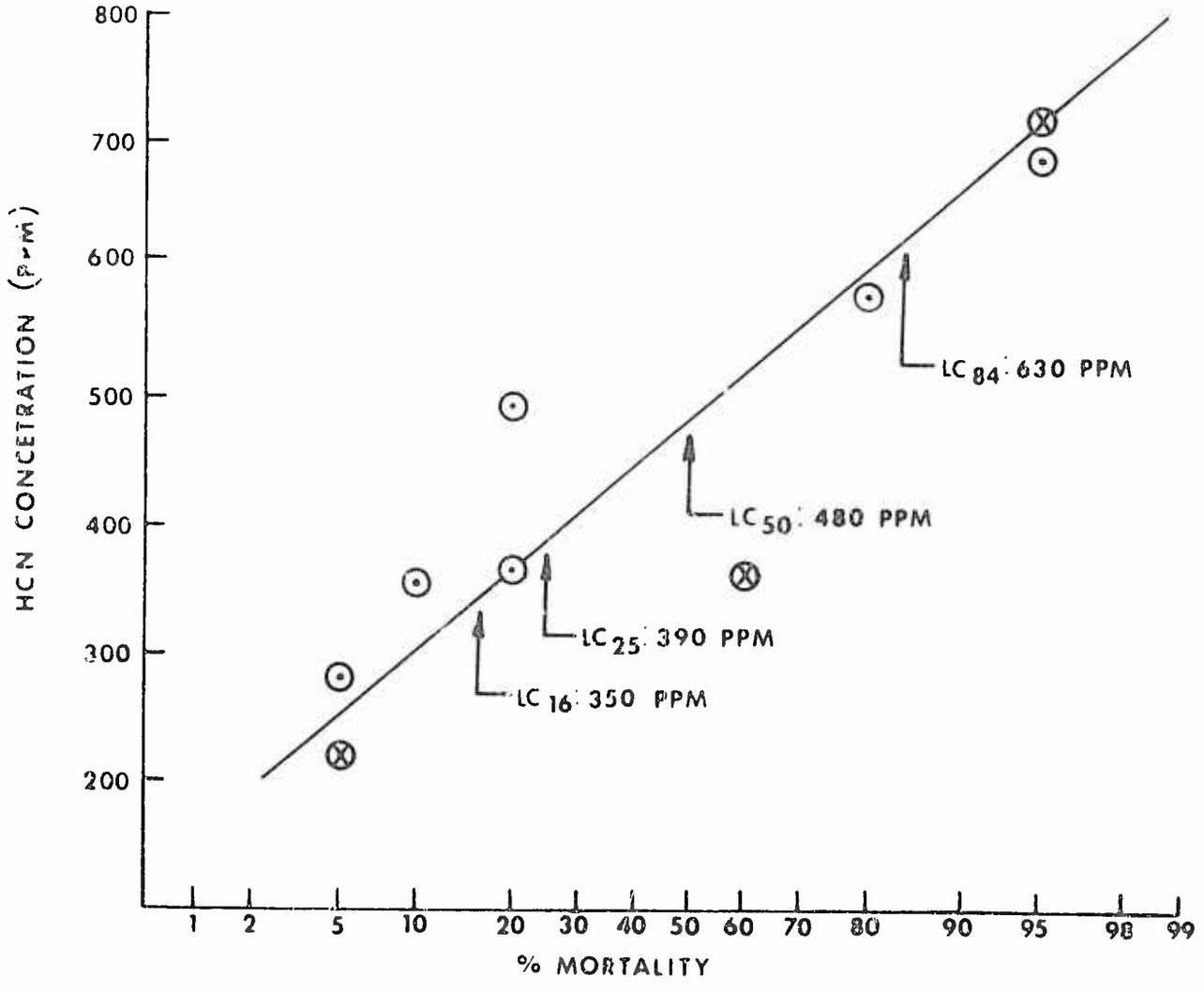


Figure 13. Rat mortality from 5-minute exposure to hydrogen cyanide.

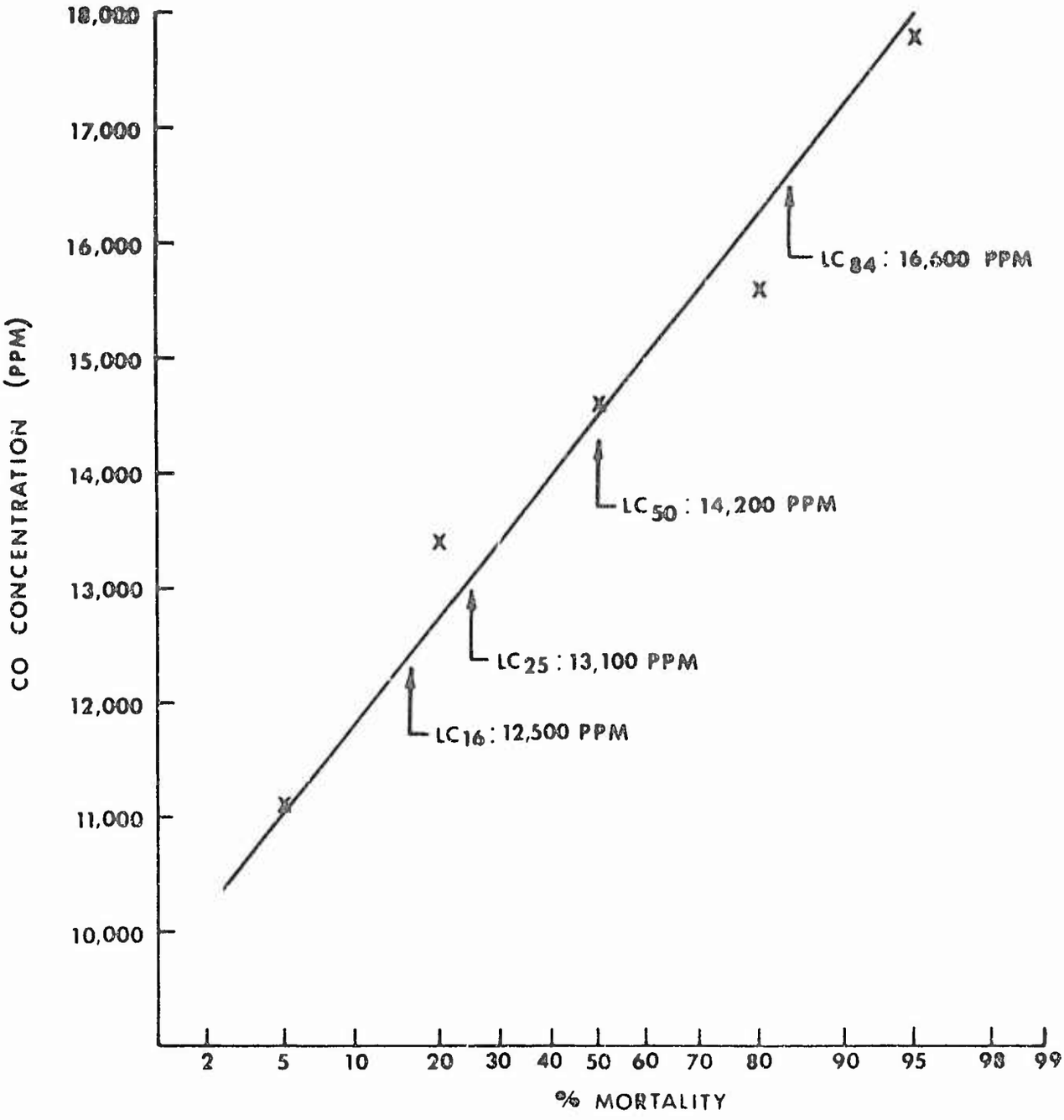


Figure 14. Rat mortality from 5-minute exposure to carbon monoxide.

For the combination exposures, 5 groups of 10 rats each were exposed to each selected combination of HCN and CO. Table 12 shows the first group of combinations. The 5-minute LC_{50} of CO was combined with the 5-minute LC_{16}, LC_{50} and LC_{84} of HCN, and the 5-minute LC_{50} of HCN was also combined with the 5-minute LC_{16}, LC_{50} and LC_{84} of CO. Each of these combinations was used for exposure of 50 rats for 2.5 minutes. Table 13 shows the second group of combinations. This consisted of exposing 50 rats to the 5-minute LC_{16} of HCN with the LC_{25} of CO for 1.5, 2.5 and 3 minutes, the two LC_{25} concentrations for 2.5, 4 and 5 minutes, and the HCN LC_{25} with the CO LC_{16} for 2.5, 3.5 and 5 minutes.

TABLE 12. CONCENTRATIONS OF HCN AND CO USED IN SIMULTANEOUS COMBINATION EXPOSURES OF RATS (N = 50)

Exposure Duration (min)	HCN Concentration (ppm)	CO Concentration (ppm)
2.5	350 (LC_{16} 5 min)	14,200 (LC_{50} 5 min)
2.5	480 (LC_{50} 5 min)	14,200 (LC_{50} 5 min)
2.5	630 (LC_{84} 5 min)	14,200 (LC_{50} 5 min)
2.5	480 (LC_{50} 5 min)	12,500 (LC_{16} 5 min)
2.5	480 (LC_{50} 5 min)	16,600 (LC_{84} 5 min)

TABLE 13. CONCENTRATIONS OF HCN AND CO USED IN SIMULTANEOUS COMBINATION EXPOSURES OF RATS (N = 50)

Exposure Duration (min)	HCN Concentration (ppm)	CO Concentration (ppm)
1.5, 2.5, 3.0	350 (LC_{16} 5 min)	13,100 (LC_{25} 5 min)
2.5, 4.0, 5.0	390 (LC_{25} 5 min)	13,100 (LC_{25} 5 min)
2.5, 3.5, 5.0	390 (LC_{25} 5 min)	12,500 (LC_{16} 5 min)

The first part of this study not only yielded information about rates of uptake of the two compounds into blood, but also was valuable for information regarding exposure to each compound individually. Since one of the objectives of this study was to determine blood values of each compound separately that would cause death, the uptake exposures provided that information. A value of 12.2 μg/ml for blood cyanide represented the highest blood cyanide level observed for the entire study. The highest COHb level found during the study was 77% and every rat that reached that level died during exposure. The data also show an interesting fact about the two compounds; the lethal range for CO alone was very narrow, while that for HCN was much wider.

The rate of uptake of HCN into rat blood is shown in Figure 15. The dose is represented as concentration times minutes of exposure (CT) in terms of ppm-minutes and the response in terms of μgCN/ml in blood. A rapid, almost linear, rise up to about 6 μg/ml was observed which then became more curved or exponential. A CT dose of about 1000 ppm-minutes was required to reach the minimum lethal blood cyanide level.

The rate of uptake of CO into rat blood is shown in Figure 16. Again, the dose is represented in terms of ppm-minutes and the blood CO level is in % COHb. The initial rate of CO uptake into rat blood was extremely fast as would be expected. Exposure to the 5-minute LC_2 concentration (10,000 ppm) for 5 minutes resulted in a COHb level of 57%. Deaths from CO occurred in a very narrow range of 72-77% COHb.

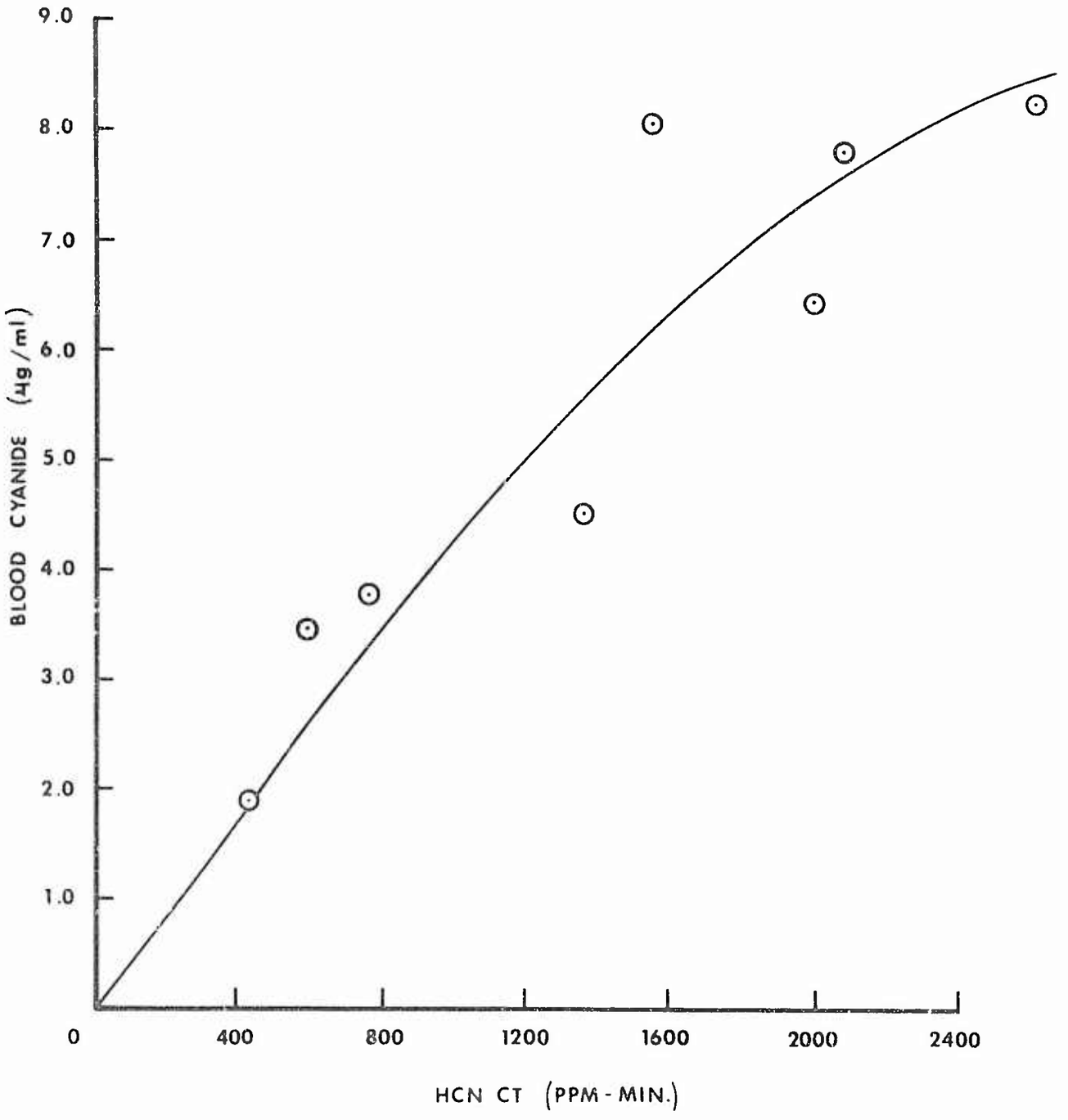


Figure 15. Hydrogen cyanide uptake into rat blood.

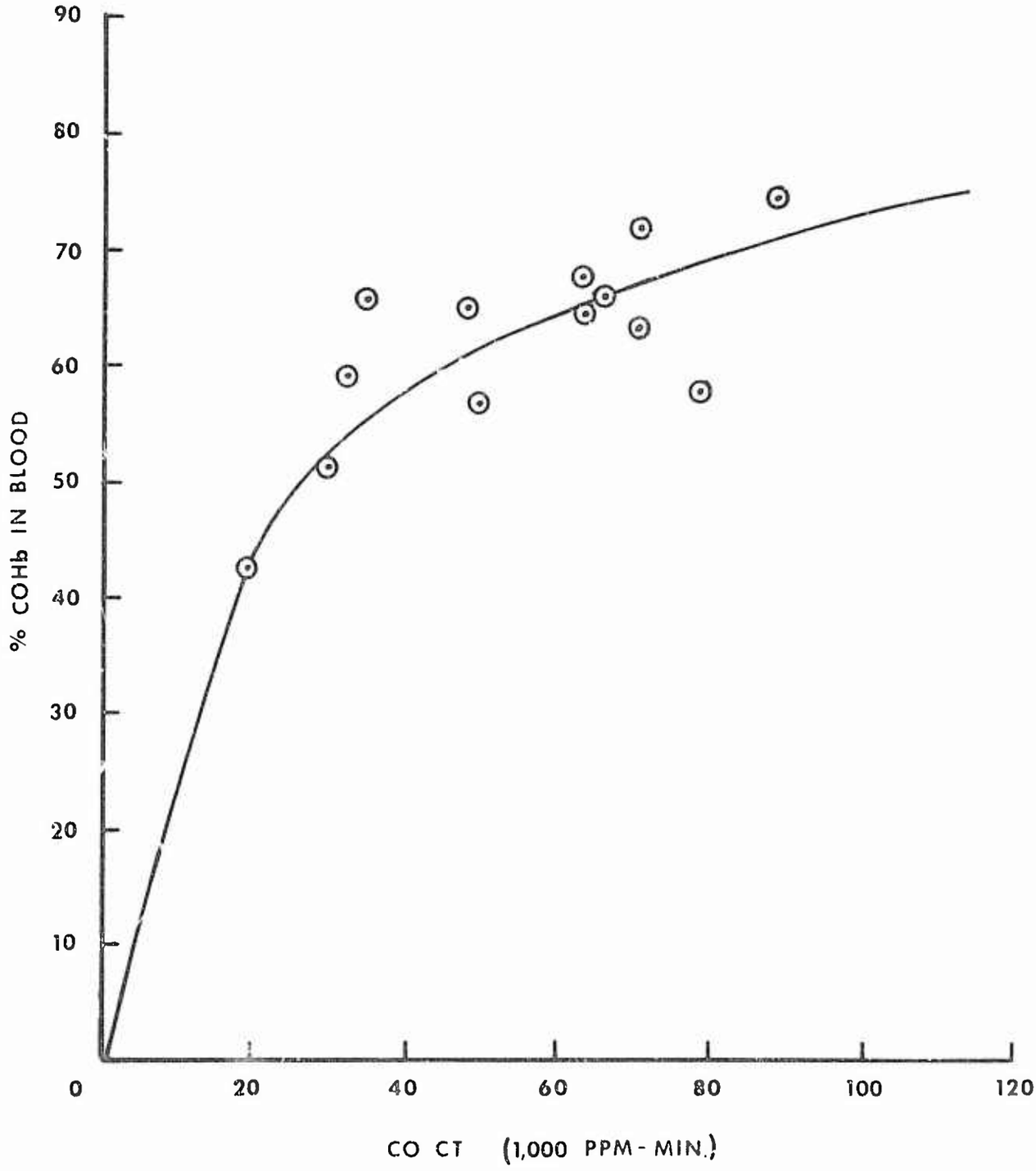


Figure 16. Carbon monoxide uptake into rat blood.

The results of combination HCN and CO exposures are still being evaluated. During the course of conducting exposures it became apparent that the variability of CN^- recovery from the blood was greater than expected from data developed in singly exposed animals. It was necessary to group results of analyses from animals in each test run to minimize the effect of this variable. The result of this grouping has led to the tentative conclusion that the toxic responses to HCN and CO are independent in action although occasional groups (4 of 59 test runs) appear to suggest some interaction.

Further experiments will be conducted on the method of cyanide analysis and additional animal exposures will be performed to clarify these tentative conclusions.

Coal Tar Volatiles Study

The 90-day continuous exposure of animals to various concentrations of coal tar aerosol started during fiscal year 1972 was completed. The experimental design and method of generation were detailed in the last annual report (MacEwen and Vernot, 1972).

An aerosol particle size determination was performed on each chamber at the beginning of the study and once monthly thereafter on the Longley Chambers only. Table 14 shows the monthly particle size characterization expressed as a percentage of droplets five microns or less in diameter. Except for chamber D-2 in the second month, all samples showed more than 95% of the droplets five microns or less in diameter.

TABLE 14. MONTHLY PARTICLE SIZE CHARACTERIZATION; 90-DAY EXPOSURE TO COAL TAR AEROSOL

(Percentage of Droplets 5 Microns or Below in Diameter)

Chamber and Aerosol Concentration	1st Month	2nd Month	3rd Month
A - 20 mg/m^3	100.0	-	-
B - 20 mg/m^3	100.0	-	-
C-1 - 20 mg/m^3	98.7	96.4	97.9
C-2 - 10 mg/m^3	98.8	95.1	95.2
D-1 - 2 mg/m^3	98.7	98.4	99.4
D-2 - 0.2 mg/m^3	97.9	92.1	98.9

Ten percent of the hamsters, weanling rats and yearling rats from the 20.0 mg/m^3 and control groups were sacrificed at the termination of exposures. The surviving animals are being observed for the remainder of their lifetime. Differential blood cell counts are being performed on the surviving test rabbits with an equal number of controls, along with 10 test and 10 control rats, for the remainder of their lifetime.

At the conclusion of the exposure period, the animals from the 20, 10 and 0.2 mg/m^3 concentrations showed a considerable accumulation of coal tar on their fur. The accumulation appeared to be dose related in its intensity with the 20 mg/m^3 animals being quite brown. Through preening and grooming, most of the discoloration had disappeared by one month postexposure.

The daily fluorometric analysis (Table 15) yielded 90-day mean concentrations of 20, 19 and 20 mg/m^3 in the three nominal 20 mg/m^3 chambers, and 10, 1.9 and 0.22 in the nominal 10, 2 and 0.2 mg/m^3 chambers.

TABLE 15. SUMMARY OF 90-DAY COAL TAR AEROSOL CONCENTRATIONS

Chamber	Nominal Concentration	90-Day Average Measured Concentration
Rochester A	20	20
Rochester B	20	19
Longley C-1	20	20
Longley C-2	10	10
Longley D-1	2.0	1.9
Longley D-2	0.2	0.22

Cumulative animal mortality showed a general graded response proportional to exposure concentration. Exceptions to this were in the ICR mice exposed to 0.2 mg/m^3 and the control male weanling rats. Both groups showed a surge in mortality during the second and third month of exposure which appeared to be due to an unidentified infection.

All rabbits which were housed beneath hamsters died within the first 67 days while only three of the 12 rabbits housed beneath rats and mice died during the 90-day exposure. In order to investigate the possibility that this arrangement is deleterious to rabbits, we housed hamsters above four control rabbits. Three of the four rabbits died within a month's time. We do not attribute the exposed rabbit deaths to coal tar exposure and we are investigating the noncompatibility of these species at the present time.

In every species, there was a marked effect of exposure on growth during the exposure period. In most cases, the difference was dose related and can still be observed seven months postexposure. Typical of the effects on animal growth are the results shown in Figures 17 through 20. With the exception of the 0.2 mg/m^3 yearling rat group, all exposure groups of every species showed a statistically significant difference in mean body weight when compared to their controls at the conclusion of the 90-day exposure period.

Measurements of fluorescent materials extracted from animal tissue with toluene showed considerable increase in lung burden after 30 days exposure to 20 mg/m^3 as shown in Table 16. There appears to be an increase in the fluorescent content of lung tissue essentially proportional to animal size.

Monthly differential blood cell counts showed no differences between the exposed animals and the controls.

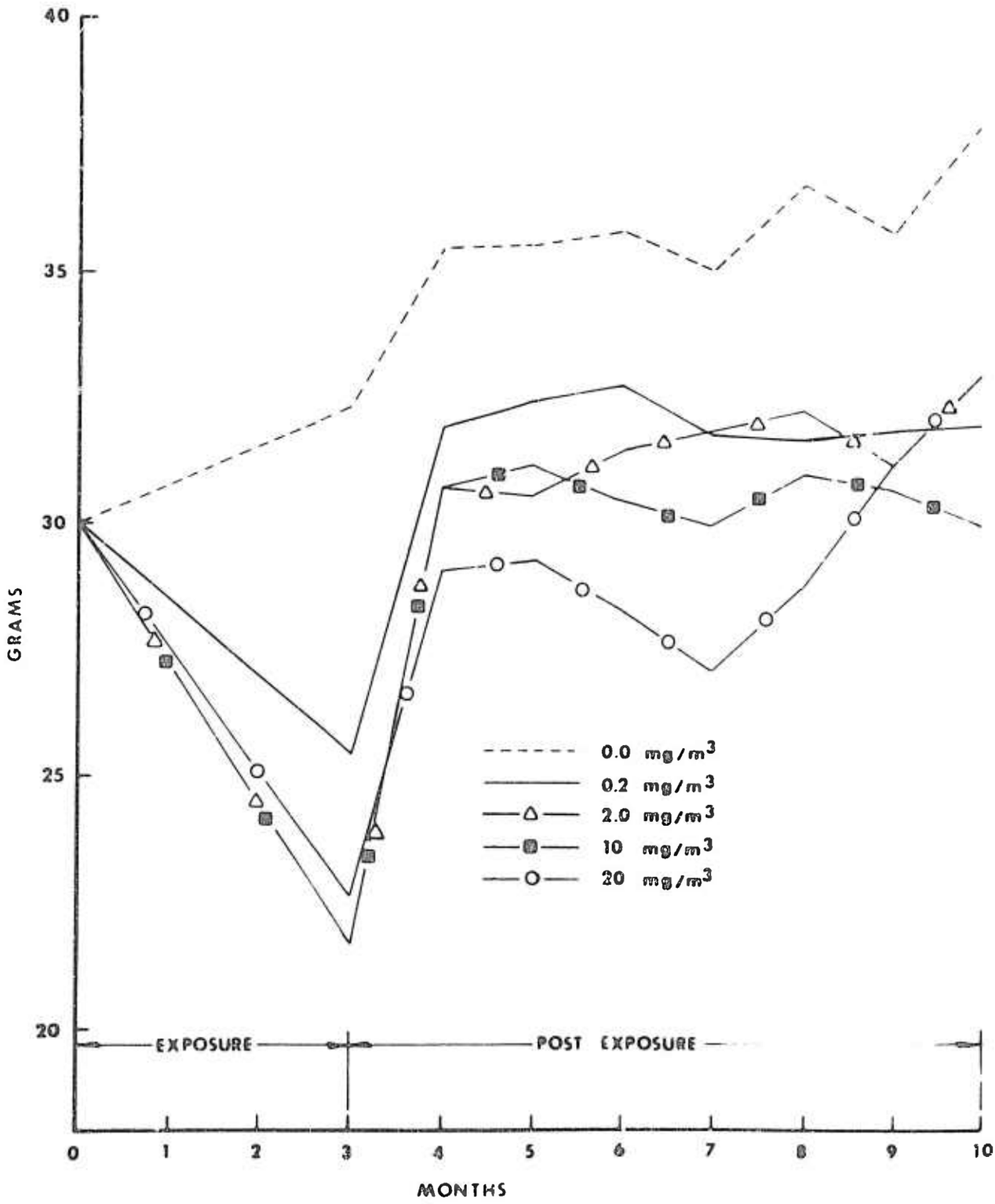


Figure 17. Growth of male CAF-1 mice exposed to a coal tar volatiles aerosol.

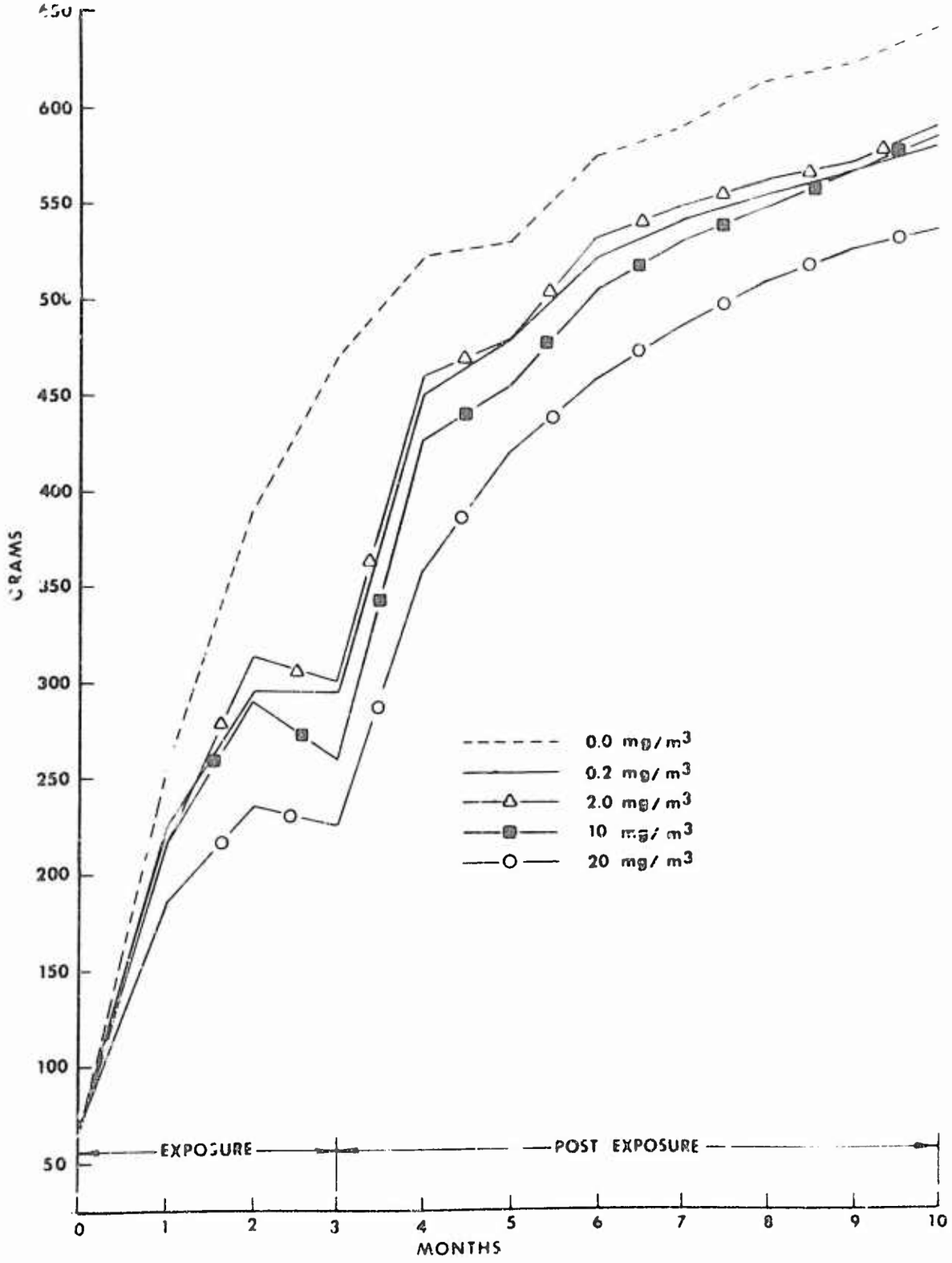


Figure 18. Growth of male weanling rats exposed to a coal tar volatiles aerosol.

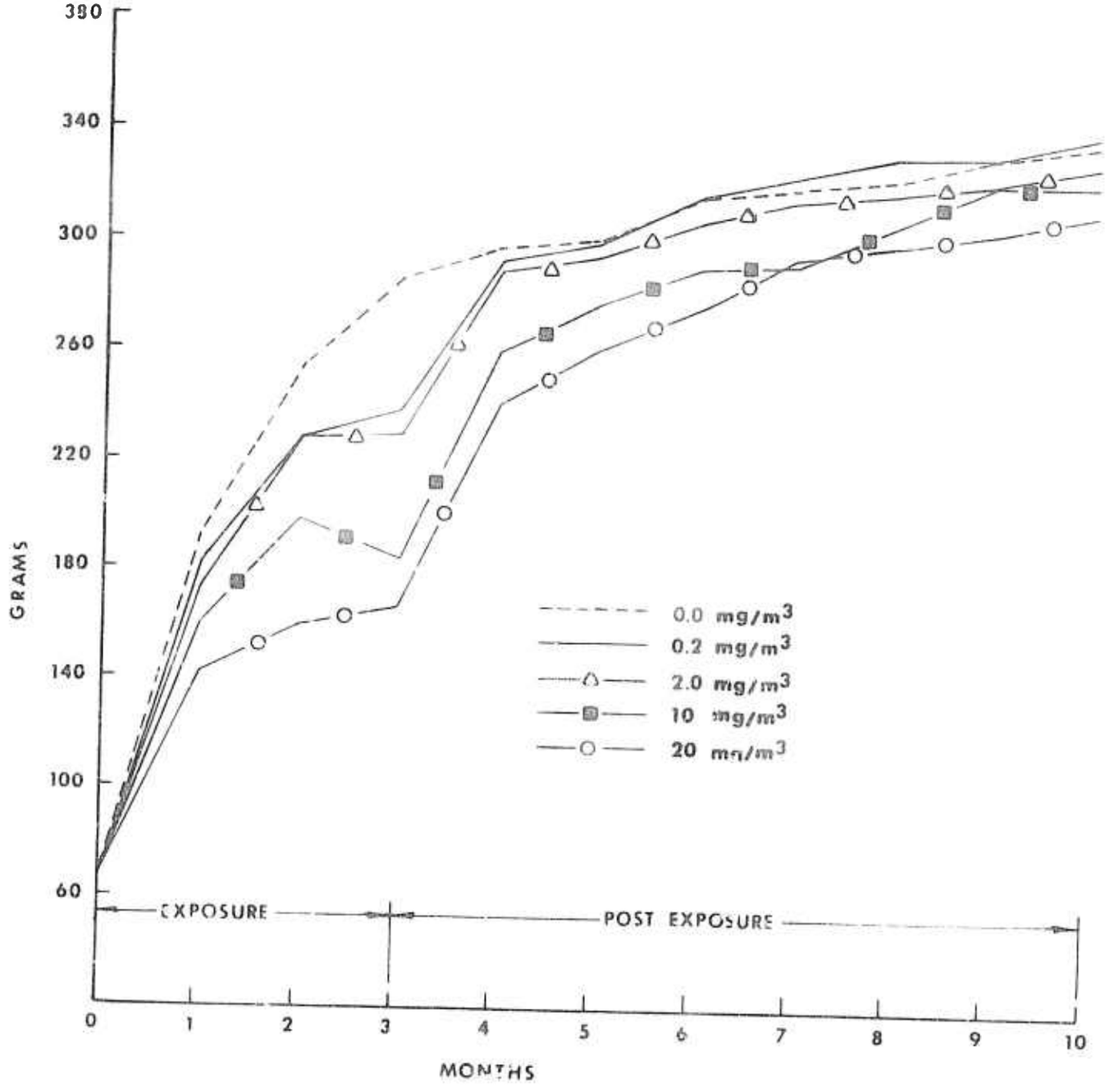


Figure 19. Growth of female rats, exposed as weanlings, to coal tar aerosol for 90 days.

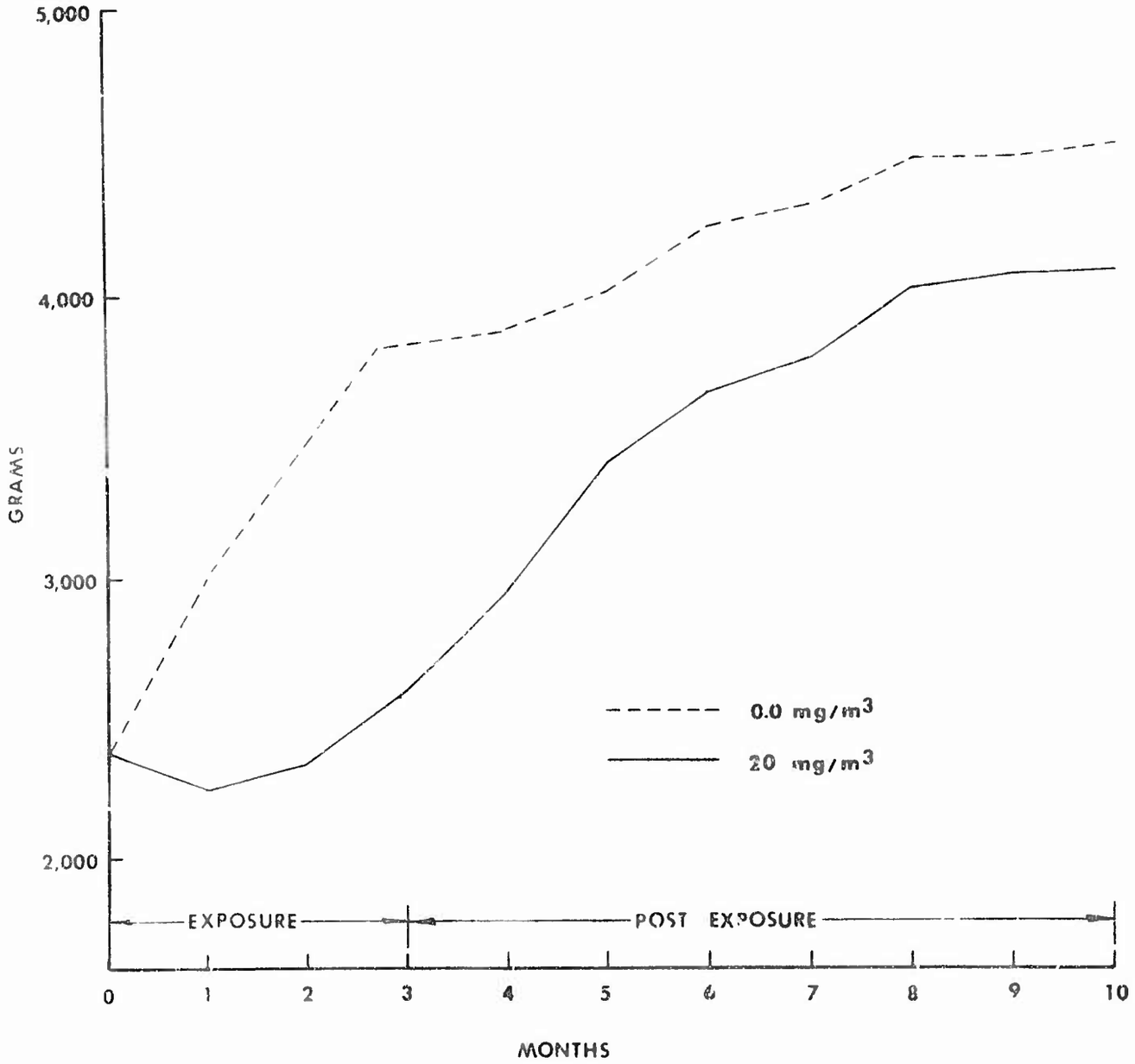


Figure 20. Growth of female rabbits exposed to a coal tar volatiles aerosol.

TABLE 16. TOLUENE SOLUBLE FLUORESCENCE IN ANIMAL TISSUE AFTER 30-DAYS CONTINUOUS EXPOSURE TO 20 mg/m^3 COAL TAR AEROSOL

		mg*/g Wet Tissue		
		Kidney	Liver	Lung
Rat:	Exposed	5.4	174	365
	Control	3.5	100	57
Mouse:	Exposed	9.7	234	253
	Control	3.1	154	4
Hamster:	Exposed	6.5	60	813
	Control	3.1	43	26
Rabbit:	Exposed	5.8	21	1003
	Control	2.7	9	5

*Coal Tar Equivalent

The inhalation of coal tar aerosols appears to stimulate proliferation of alveolar macrophages in the lung which contain the material in the form of microglobules, brownish-black in color. Most of these macrophages are found within the lumen of the alveoli or attached to the alveolar wall. A lesser number were found in the interstitium and in peribronchial lymphoid areas. Their numbers were proportional to the exposure level.

Several mice in the experimental group had focal accumulations of eosinophilic granular cells which resembled granular pneumocytes or possibly macrophages in the air spaces and adjacent interstitium. It appeared to be an area of resolving pneumonitis. In one animal there was a suggestion of metaplasia. It did not appear to be directly related to the coal tar since pigment was not conspicuous and the lesion was found in the controls in about the same frequency.

Adenomas of the lung were found in 3 of 30 controls and 4 of 27 high level (20 mg/m^3) experimental mice. All were identical in their microscopic morphology. They were usually in a peripheral location, were well circumscribed but not encapsulated, spherical in shape and were growing by expansion with mild compression of the surrounding parenchyma. They were true adenomas which were composed of compact papillary cords supported by minimal fibrovascular stroma. The essential cell was cuboidal with a slightly hyperchromatic oval reticulate nucleus. The appearance resembled bronchiolar epithelium.

Gross examination of the animals for skin lesions by a qualified pathologist was first done 90 days postexposure. At this time several mice from the 20 mg/m^3 group showed skin tumors (see Table 17). While most tumors were found in the 20 mg/m^3 group, a few mice in the 10 mg/m^3 group and one in the 2 mg/m^3 also showed tumors. New tumors were found in subsequent examinations until 8 March 1973. No new tumors have been found since that time.

Macroscopically the tumors varied from small papilloma like growths with a narrow base to larger sessile tumors with a central core of keratin. Ulceration and inflammation was a common feature at the edge of the larger growths. Growth was usually continuous with no indication of spontaneous regression. There was no evidence of metastasis to regional lymph nodes

TABLE 17. CUMULATIVE MOUSE SKIN TUMORS OCCURRING AFTER COAL TAR AEROSOL EXPOSURE

Date Examined	Mouse Strain and Concentration, mg/m^3									
	ICR 20	JAX 20	ICR 10	JAX 10	ICR 2	JAX 2	ICR 0.2	JAX 0.2	ICR 0	JAX 0
9 Nov. 1972	2*	6	0	0	0	0	0	0	0	0
13 Dec. 1972	6	7	1	0	0	0	0	0	?	0
2 Jan. 1973	6	7	1	0	1	0	0	0	0	0
24 Jan. 1973	7	7	2	0	1	0	0	0	0	0
9 Feb. 1973	7	8	2	0	1	0	0	0	0	0
22 Feb. 1973	9	8	3	0	1	0	0	0	?	0
8 Mar. 1973	9	8	3	0	1	0	?	?	?	0
22 Mar. 1973	9	8	3	0	1	0	?	?	0	0
6 Apr. 1973	9	8	3	0	1	?	?	?	0	0
20 Apr. 1973	9	8	3	0	1	?	?	?	0	0
3 May 1973	9	8	3	0	1	0	?	?	0	0
15 May 1973	9	8	3	0	1	0	0	?	0	0

* Number of animals examined were unequal and decreasing with time.

? Questionable lesion.

or other organs, even with the largest tumors. Figure 21 shows an ICR mouse with a typical tumor on the base of the ear. Note the wide base and hyperkeratotic appearance. Figure 22 is another ICR mouse with an extremely large tumor which has destroyed the ear and adjacent tissues. The central horn-like mass composed almost entirely of keratin.

Microscopic examination was usually confined to tumors from animals that died spontaneously. The histopathology varied from benign squamous papillomas to keratoachanthoma to squamous cell carcinoma with by far the most cases falling into the latter category. The more aggressive features were characterized by nuclear atypia, increased mitosis with bizarre figures, aggressive downward growth through the basement membranes into the adjacent fibrous connective tissues and skeletal muscles, spindle cell formation in the latter areas, keratin pearl formation and inflammation of adjacent connective tissues. Invasion of lymphatics and blood vessels was not observed in any case. Surface erosion, ulceration and associated purulent response was a common feature. The central portion of many of the tumors contained voluminous amounts of keratin.

A single skin tumor was observed in a rat exposed to 20 mg/m^3. Microscopically it appeared to be a fairly aggressive squamous cell carcinoma. No evidence of metastasis was observed. A single, small papilloma-like lesion was found in the ear of an exposed rabbit (20 mg/m^3). It has shown no tendency to grow since it was first observed.

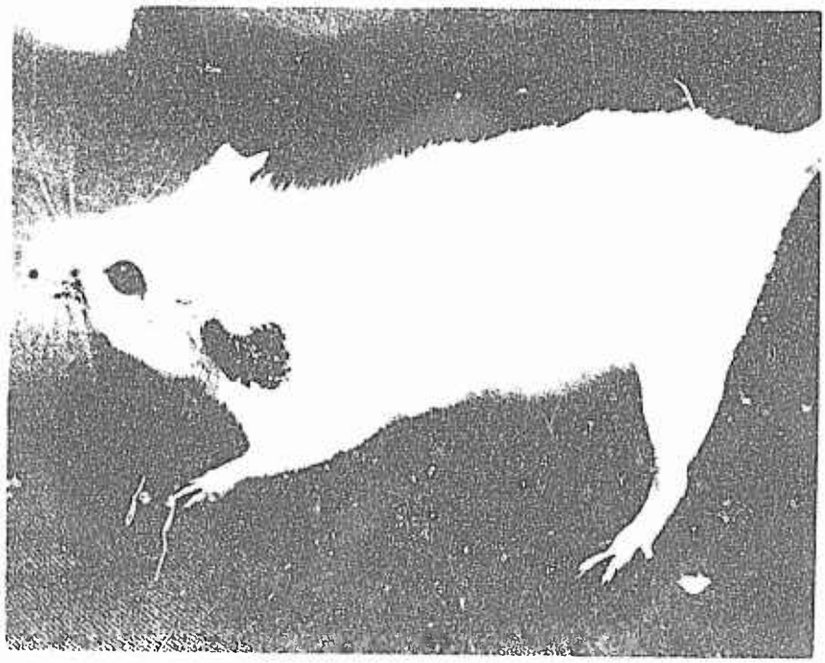


Figure 21. Coal tar exposed mouse with typical tumor of ear.

Figure 22. Coal tar exposed mouse with enlarged tumor of head and neck.

The liver from one control mouse showed focal nodular hyperplasia which may be a preneoplastic change. Neoplastic changes or other changes associated with the contaminant were not found in any other organ.

Based on the data accumulated to this point, several tentative conclusions can be made:

1. Coal tar appears to be a skin carcinogen for mice.
2. This potential appears to be both dose related and time related.
3. Skin tumorogenic activity is markedly less in rats and non-existent in hamsters and rabbits.
4. Tumors once started do not regress.
5. A wide range of skin tumors are encountered. However, if allowed to progress most appear to become squamous cell carcinomas.
6. The tumors do not metastasize, even those that appear aggressive macro and microscopically.

Coal Tar Volatiles Study II

The preliminary study of the toxicity of coal tar volatile materials from coke oven effluent was conducted on four animal species for a 90-day period under continuous inhalation exposure conditions. Due to difficulties in the generation of an aerosol of the coal tar, solids were removed to reduce viscosity, and the benzene (1:1) soluble fraction of coal tar was used as the exposure material.

The coal tar used in the preliminary study did not contain a light oil fraction of the coke oven distillate which is normally removed late in the separation process. This light oil fraction is also known as the BTX fraction and consists primarily of benzene, toluene and xylene.

The animal exposures were conducted in an attempt to reproduce the increased incidence of pulmonary carcinogenesis in coke oven workers as reported by Lloyd (1971) and consequently, an ideal study would require inhalation exposure to the entire complex mixture of coke oven effluent to avoid the possibility of eliminating the active agent or agents responsible for cancer induction. The coal tar used in this study contains both the solids and the BTX fraction.

The animals are being exposed continuously for 90 days to the aerosolized coal tar volatiles at a concentration of 10 mg/m^3. A control group of each species is being maintained for comparison with the test animals. The animals are observed daily for general appearance, behavior, signs of toxic stress and lethality.

Experimental animals include female Sprague-Dawley yearling rats, male and female Sprague-Dawley weanling rats, male CF-1 mice, male CAF-1 mice, male Golden Syrian hamsters and female New Zealand white rabbits. The animal complement and chamber loads are as follows:

1. Test Animals

Two Longley Chambers

80 Yearling rats (all females)

80 Weanling rats (40 males, 40 females)

150 Mice, male (75 CAF-1, 75 CF-1)

100 Golden Syrian Hamsters (male)

Two Rochester Chambers

12 New Zealand white rabbits (female)

150 CF-1 mice (male)

2. Control Animals

80 Yearling rats (female)

80 Weanling rats (40 male, 40 female)

300 Mice, males (225 CF-1, 75 CAF-1)

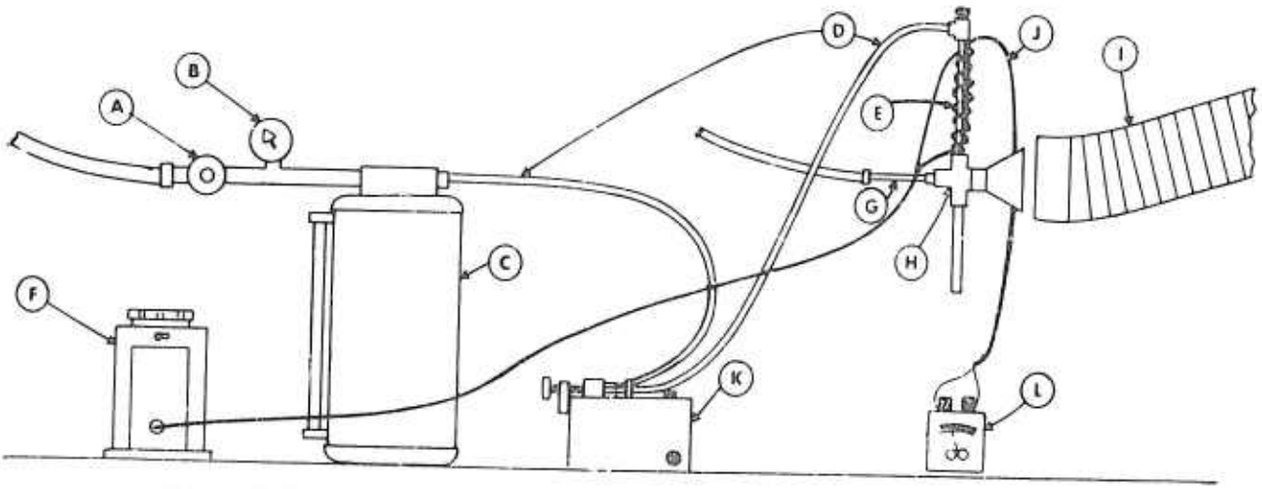
100 Golden Syrian hamsters (male)

12 New Zealand white rabbits (female)

Ten percent of the hamsters, weanling rats and yearling rats from the test and the control groups will be sacrificed at the termination of exposures. The remaining animals will be observed for a minimum of 37 additional months before being sacrificed. All animals that died are being submitted to the veterinarians for gross pathological examination. In addition, any animal that dies will have sections of kidney, liver and lung submitted to chemistry for measurement of fluorescent compounds. Histologic

examination will include liver, lung, genito-urinary tract (including bladder), scrotum, skin, spleen and bone marrow. Once per month five of the test CF-1 mice from the Rochester chamber and five controls are randomly sacrificed and submitted to the pathology branch for gross and histological examination of pulmonary pathology. These mice will have a 1" x 1" patch of skin (fur intact) removed from the back and a section of lung submitted for analysis of fluorescent compounds. During the postexposure observation period, complete hematology with indices will be performed on 10 rabbits and 10 rats each month (test and controls).

Some modifications have been made in the generation system for this study which is pictured in Figure 23. The actual generating device, a 1/8" stainless steel "cross" pipe fitting, has remained the same. Between the coal tar reservoir and the generator is a Buchler Polystaltic® pump which is used to regulate the amount of coal tar entering the generating device. Polyethylene tubing, 1/8" O.D., carries the coal tar to the pump and from the pump to the generator. Technicon Acidflex® transmission tubing is used to carry the coal tar through the polystaltic pump. A slight pressure is maintained on the reservoir to prevent collapsing of the transmission tubing. The chamber concentration can be regulated by controlling the speed of the polystaltic pump. Prior to entering the generator, the delivery line is subjected to mild heat (65 C) to facilitate the aerosolization process.



(A) Coal Tar Pressure Regulator
(B) Coal Tar Pressure Gauge
(C) Coal Tar Reservoir
(D) Coal Tar Supply Line
(E) Coal Tar Delivery Line Heated with Nichrome Wire
(F) Variable Output Transformer
(G) Generator Air Line
(H) Generator Nozzle
(I) Chamber Aerosol Delivery Line
(J) Thermocouple
(K) Buchler Polystaltic Pump
(L) Pyrometer

Figure 23. Contaminant generation system for aerosolization of coal tar volatiles.

The chamber concentrations are analyzed using gravimetric sampling to trap the aerosol droplets on a millipore filter. The fluorescent materials are dissolved from the filter with toluene and the fluorescence measured by a Turner fluorometer. Sampling was done hourly during the first two weeks and every two hours thereafter when it was determined that the concentrations were stable.

An aerosol particle size determination was performed on each chamber at the beginning of the study following the procedure of Vooren and Meyer (1971). Table 18 shows the results of this initial droplet size determination. A minimum of 97% of the total droplets in all chambers are five microns or less in diameter. Additional particle size determinations will be made monthly during the remainder of the study.

Monthly body weights are measured on the weanling rats and the rabbits during the course of the study as both species are in the rapid growth phase and should best demonstrate any adverse exposure effects on normal growth rate. Table 19 shows the result of the first month's weighings of these animals. It can clearly be seen that the treatment has had an adverse effect on the animals as each exposed group has shown a statistically significant effect on the mean body weight gain. These animals are showing a weight-gain suppression very similar to what was seen in the animals during the previous coal tar study.

TABLE 18. PARTICLE SIZE DISTRIBUTION OF COAL TAR AEROSOL SAMPLED DURING THE FIRST MONTH OF A 90-DAY STUDY

Chamber	Droplet Size in Microns	Number of Particles	Percentage of Particles	Cumulative Percentage of Particles
A	< 2	277	84.7	84.7
	2-5	36	11.0	95.7
	5-10	14	4.3	100.0
B	< 2	271	86.4	86.4
	2-5	35	11.1	97.5
	5-10	8	2.5	100.0
C	< 2	266	84.7	84.7
	2-5	33	10.5	95.2
	5-10	15	4.8	100.0
D	< 2	310	86.6	86.6
	2-5	36	10.0	96.6
	5-10	12	3.4	100.0

TABLE 19. MEAN WEIGHT GAIN OF RABBITS AND WEANLING RATS AFTER ONE MONTH EXPOSURE OF 10 mg/m^3 COAL TAR AEROSOL

Species and Sex	Treatment	Mean Weights, gms. 0 Days	Mean Weights, gms. 1 Month	Mean Weight Gain, Grams
Rabbit, ♀	Exposed	2370	2760*	390
Rabbit, ♀	Control	2290	3060	770
Weanling Rats, ♂	Exposed	44	190*	146
Weanling Rats, ♂	Control	44	253	209
Weanling Rats, ♀	Exposed	41	139*	98
Weanling Rats, ♀	Control	41	175	133

*Different from controls at 0.01 level of significance.

In addition to the 150 CF-1 mice previously mentioned to be used for monthly serial sacrifice, 10 were included for 24-hour and 1-week examinations. Table 20 shows the results of examinations of lung tissue and hide (skin and fur) for fluorescent compounds. A time dose response can be seen in the results of the lung fluorescence. This is also seen in the hide fluorescent results after 24 hours and one week. However, the values at one month show less fluorescence than after 24 hours. We have not yet determined whether there is something wrong with the method used or if this difference is real. This should be resolved with the additional experience examining hide fluorescence during the remainder of the study.

The exposures are interrupted approximately 15 minutes each day for routine maintenance. All leftover animal food is discarded and replaced with a fresh supply. The cages in the Longley chambers are rotated daily, the top cage going to the bottom and all other cages moved up one level. The animal cages are changed on a weekly schedule.

The inhalation exposures are now in progress and will be completed during the next report period.

TABLE 20. SERIAL FLUORESCENCE MEASUREMENTS OF MOUSE HIDE AND LUNG TISSUES DURING EXPOSURE TO COAL TAR AEROSOL

Mouse Lung Fluorescence
(Expressed as μg Coal Tar/g Lung Tissue)

Mouse Number	1	2	3	4	5	Ave.	Ave. Less Control Value
Control	65	48	65	64	78	64	
24 Hours	85	80	97	102	113	95	31
7 Days	272	296	235	242	294	268	204
Control	72	97	79	82	88	84	
30 Days	830	615	855	900	560	752	668

Mouse Hide Fluorescence
(Expressed as Mean Group Values, N=5)

Treatment, Time	cm^2 area	wt. gms.	μg/g	$\mu g/cm^2$
Controls	13.80	1.493	0	0
Exposed, 24 hours	10.11	0.958	284	26.9
Exposed, 7 days	12.85	1.235	366	34.9
Controls, 1 month	9.01	1.415	0.56	0.089
Exposed, 1 month	8.52	0.934	178	19.2

Preliminary Monomethylhydrazine-Drinking Water Studies

Carcinogenic effects of MMH on hamsters and Swiss mice have been recently reported by Toth (1972 and 1973). In one study, solutions of 0.001% hydrazine, 0.01% methylhydrazine and 0.001% methylhydrazine sulfate were administered continuously in the drinking water of groups of 5- and 6-week old randomly bred Swiss mice for their entire lifetime. Hydrazine and methylhydrazine sulfate significantly increased the incidence of lung tumors (adenomas) in Swiss mice, while methylhydrazine enhanced the development of this neoplasm by shortening its latent period. In the second study of Toth, Syrian Golden hamsters received 0.01% methylhydrazine in drinking water daily ad libitum for life. Malignant histocytomas (Kupffer cell sarcomas) were seen in the livers of 43% of the treated animals, while none were observed in the untreated control groups.

These studies represent the first indication that MMH may be carcinogenic and therefore a more serious occupational exposure hazard then previous work had indicated. There were, however, two baffling points of concern with these studies that caused further review; (1) the calculated daily dose of MMH given in drinking water had been shown to produce acute toxic responses in rodents and other animal species if given by the inhalation or

injection routes of administration in pure form and (2) other studies had shown that MMH was very unstable in the presence of oxygen or air and it was therefore probable that the animals were not exposed to MMH (at least not a 0.01% solution) but to its oxidation products which are generally considered nontoxic.

In view of these considerations studies were planned to test the reproducibility of the findings of carcinogenic effects while monitoring the MMH content of the drinking water. Preliminary experiments concerned with the stability of MMH in water were conducted and are reported here.

Using an MMH-water solution, oral LD_{50}'s were determined for female yearling rats, male weanling rats and male hamsters. The MMH solution consisted of distilled water, Macllwains Buffer and glacial acetic acid in a 100:10:2 ratio; the total solution being 200:1 with respect to MMH content. This mixture provided a nominal MMH concentration of 4.39 μg/ml, with a pH of 4.8.

Animals were treated on a body weight basis, with a geometric progression factor of 1.26 between dose levels to facilitate the use of Weil's moving average method of LD_{50} determination (Weil, 1952). Five animals were used per dose level with three dose levels per experimental determination. Table 21 presents mortality results for each of the species tested. The comparative LD_{50} values and 95% confidence limits obtained are shown in Table 22.

TABLE 21. MORTALITY RESPONSE OF YEARLING RATS, WEANLING RATS AND HAMSTERS TO ORAL DOSES OF MONOMETHYLHYDRAZINE

Species	Dose (mg/kg)	(No. Died/No. Dosed
Yearling Rats (Weight Range 330-490 gm)	32	2/5
	40	3/5
	50	5/5
Weanling Rats (Weight Range 90-120 gm)	32	1/5
	40	2/5
	50	4/5
Hamsters (Weight Range 76-103 gm)	25	3/5
	32	4/5
	40	5/5

TABLE 22. ORAL LD_{50} VALUES AND 95% CONFIDENCE LIMITS FOR ANIMALS TREATED WITH MONOMETHYLHYDRAZINE

Species	LD_{50} Values (mg/kg)	95% Confidence Limits (mg/kg)
Yearling Rats	35.9	26.5 - 48.9
Weanling Rats	41.9	32.5 - 53.9
Hamsters	25.8	22.3 - 29.8

In addition to the oral LD_{50} determinations, a five day oral dosing study was performed on a group of five hamsters. The same buffered MMH solution utilized for the LD_{50} tests was used for these purposes. Animals were dosed four times daily (0800, 1200, 1600 and 2000 hours) to provide a total daily dose of 16 mg/kg. During the five day period, no abnormal behavior or symptomatology was noted. However, 32 hours after the last dose was administered, one of the animals was found dead, with no apparent sign of convulsive activity. Gross pathological examination of the animal indicated the presence of a fatty liver.

Due to instability of the MMH drinking water solution to be used for the proposed extended ingestion study, it was found necessary to adjust the pH of the solution to between 3.5-4.5. This afforded stability for at least a 24-hour period. Because of the possibility that the lowered pH might lead to decreased water and food consumption, a test was devised to determine the effect of lowered pH on water consumption and weight gain in hamsters. The pH was adjusted by using either citric acid or HCl additions to drinking solutions. Results are shown in Table 23.

Because the initial findings indicated a substantial weight loss for the citric acid group, a subsequent test was run in which the water bottles were switched from one group to the other. Thus, the group formerly receiving H_2O now received citric acid - H_2O and vice versa. Water consumption did not appear to be affected in the initial test so it was not measured in this

TABLE 23. EFFECTS OF LOWERED pH IN DRINKING WATER ON WATER CONSUMPTION AND WEIGHT GAIN IN HAMSTERS

Body Weights

	H_2O		Citric Acid - H_2O (pH 3.5)		
	Initial	5 Days		Initial	5 Days
#1	101 gm	107 gm	#1	93 gm	94 gm
#2	93 gm	99 gm	#2	112 gm	109 gm
#3	96	99 gm	#3	91 gm	92 gm
$\bar{x}$ weight gain = + 5.0 gm			$\bar{x}$ weight gain = -0.33 gm		

Drinking Solution Consumption (5 Days)

H_2O	Citric Acid - H_2O (pH 3.5)
175 ml/3 animals/5 days	171 ml/3 animals/5 days
= 58.5 ml/5 days	= 57.0 ml/5 days
= 11.7 ml/day	= 11.4 ml/day

Drinking Solution Consumption (4 Days)

H_2O	H_2O - HCl (pH 3.6)
172 ml/3 animals/4 days	162 ml/3 animals/4 days
= 54 ml/4 days	= 54 ml/4 days
= 13.5 ml/day	= 13.5 ml/day

repeat run. Results from the second trial paralleled those first observed. Citric acid, although not affecting water consumption, appears to have affected appetite suppression, possibly by serving as an alternative energy source with the exclusion of the proper nutrients needed for growth promotion.

The group treated with HCl - H_2O did not exhibit any abnormalities with respect to either weight gain or water consumption. Based on this data, the HCl induced pH's tested are not a factor influencing body weight gain or water consumption, at least not for the short periods tested.

Chlorine Pentafluoride Emergency Exposure Limits

Chlorine pentafluoride (ClF_5) has been studied extensively in our laboratory during the past three years (MacEwen and Vernot, 1972; MacEwen and Vernot, 1971; Darmer et al., 1972). The acute toxicity of ClF_5 in rats, mice, dogs and monkeys was examined and LC_{50} values for 15-, 30- and 60-minute exposures of each of these four species were established.

Exposure to ClF_5 causes immediate and severe irritation to any exposed tissue, especially if it is moist, such as the respiratory tract and the eyes. Dogs, monkeys and rodents each showed unmistakable signs of irritation almost immediately after onset of any given exposure. The respiratory tract and lungs were found to be the primary targets for ClF_5 damage.

Evidence at the gross pathological level showed that the lungs had been severely compromised and were filled with edema fluid, mucous and in some cases blood. Histopathological examination showed severe alveolar destruction and congestion. Delayed deaths were seen in all four species, which was similar to that pattern observed with other severe pulmonary irritants.

The damage inflicted by ClF_5 was seen to be partially reversible over a 2-week postexposure period. Much of the congestion and fluid accumulation had disappeared, and the lungs often appeared almost normal upon gross examination. There was, however, residual damage which was irreversible, such as scarring and consolidation of lung tissue.

With the acute toxicity picture well defined, the next question dealt with Emergency Exposure Limits (EEL's) for ClF_5. Knowing the extremely potent irritation effects of ClF_5, it was crucial to establish an EEL which would enable any personnel working with this compound to escape from an exposure area before becoming disabled by the symptomatic effects of ClF_5. The existing recommended EEL's for ClF_5 were established in 1968 by the National Academy of Sciences, Advisory Center of Toxicology by analogy with bromine pentafluoride (BrF_5), in view of the close similarity between the chemical and physical properties of the two compounds. However, at that time the existing toxicological data on BrF_5 were not considered by the Committee on Toxicology to be adequate for more than a very tentative recommendation on EEL's. The data on ClF_5 was even more incomplete than for BrF_5, so the recommended levels for ClF_5, which follow, were highly tentative:

Exposure Time	ClF_5 Concentration	C. T. (Concentration x Time)
10 min.	3.0 ppm	30 ppm-min.
30 min.	1.5 ppm	45 ppm-min.
60 min.	0.5 ppm	30 ppm-min.

Preliminary experiments in our laboratory on ClF_5 EEL's were described in last year's annual report (MacEwen and Vernot, 1972). Those exposures were to ClF_5 levels of 30 ppm for 10 minutes, 30 ppm for 30 minutes, and 10 ppm for 60 minutes; more than ten times the existing EEL levels, and about ten times lower than the lower LC_{50} values (Darmer et al., 1972). No deaths occurred during any of those exposures, but two deaths did occur during the 4-week postexposure period. These were a mouse that died at 4 days and a monkey at 6 days; both in the 30 minute-20 ppm ClF_5 group. There were slight effects on body weight in mice and monkeys and scattered significant differences between control and exposed clinical chemistry parameters. However, gross and histopathological examination of all animals at 28 days postexposure failed to produce any evidence of permanent effect of exposure to these levels of ClF_5.

In view of these results, it was felt that further investigation was essential. During this report period, additional EEL exposures were conducted. The first group of exposures for 10, 30 and 60 minutes were made using rats, mice, dogs and monkeys at an equal ppm-minute dose of 300 as described below. A second group of exposures involving rats and mice were

conducted for 10 minutes only at the same 300 ppm-minute dose level. A third group of exposures of rats only to doses of 30 and 70 ppm-minutes ClF_5 are also described.

Groups of 30 rats, 30 mice, 8 dogs and 5 monkeys were exposed to ClF_5 concentrations of 30 ppm for 10 minutes, 10 ppm for 30 minutes and 5 ppm for 60 minutes. The exposures were conducted in a modified Rochester chamber (MacEwen and Vernot, 1968) equipped with sliding gasketed cage drawers which could be rapidly inserted and withdrawn from the chamber after the desired concentration had been achieved within the chamber. Chlorine pentafluoride concentrations were monitored continuously as previously described (Darmer et al., 1972). The animals were observed for overt signs of toxicity during exposure and for 28 days postexposure with the exception of monkeys, which were followed for 6 weeks postexposure. Dogs and monkeys were bled at 14 and 28 days postexposure for clinical blood chemistry determinations, and body weight information was recorded during the postexposure period for all 4 species. A control group of the same numbers of each species was maintained for comparison with the exposed groups.

The typical ClF_5 irritation and discomfort symptoms (salivation, eye irritation, lacrimation and rhinnorhea) were seen during the exposures, with the most severe effects being observed in the 10 minute-30 ppm exposure group. One monkey from the control group was found to be in extremely

poor health 26 days after the exposures. Since death was considered to be imminent, the monkey was sacrificed and examined on that day. Gross examination revealed no lesions of any sort which would account for the condition of the monkey. A virus infection was suspected, but not substantiated. Also, one cage of 5 rats from the 30 minute-10 ppm exposure group was lost during the third week postexposure due to accidental drowning when the cage in which they were housed activated a dog lixit valve, filling it with water. These rats appeared normal up to the time of their death, and there was no reason to believe they would have died from any damage due to the exposure.

Gross pathology revealed no changes in any of the animals which could be related to the exposures. Body weight information for the four species is presented in Figures 24 through 27. Rats had perfectly normal weight patterns postexposure (Figure 24). Mice had normal weight patterns with a possible exception of the 30 minute-10 ppm group which experienced a slight weight loss between 2 and 4 weeks postexposure, but this was not enough to be significantly different from controls (Figure 25). Dog weights were completely normal (Figure 26). All monkey groups, including controls, experienced a slight weight loss postexposure due to a change in living quarters, but by 4 weeks all groups except the 30 minute-10 ppm group had begun to gain weight normally (Figure 27). The weight loss continued in the 30 minute group at 6 weeks postexposure, with the 60 minute-5 ppm group also experiencing a slight drop at 6 weeks postexposure. This may be indicative of an effect due to exposure, especially in the 30 minute-10 ppm group.

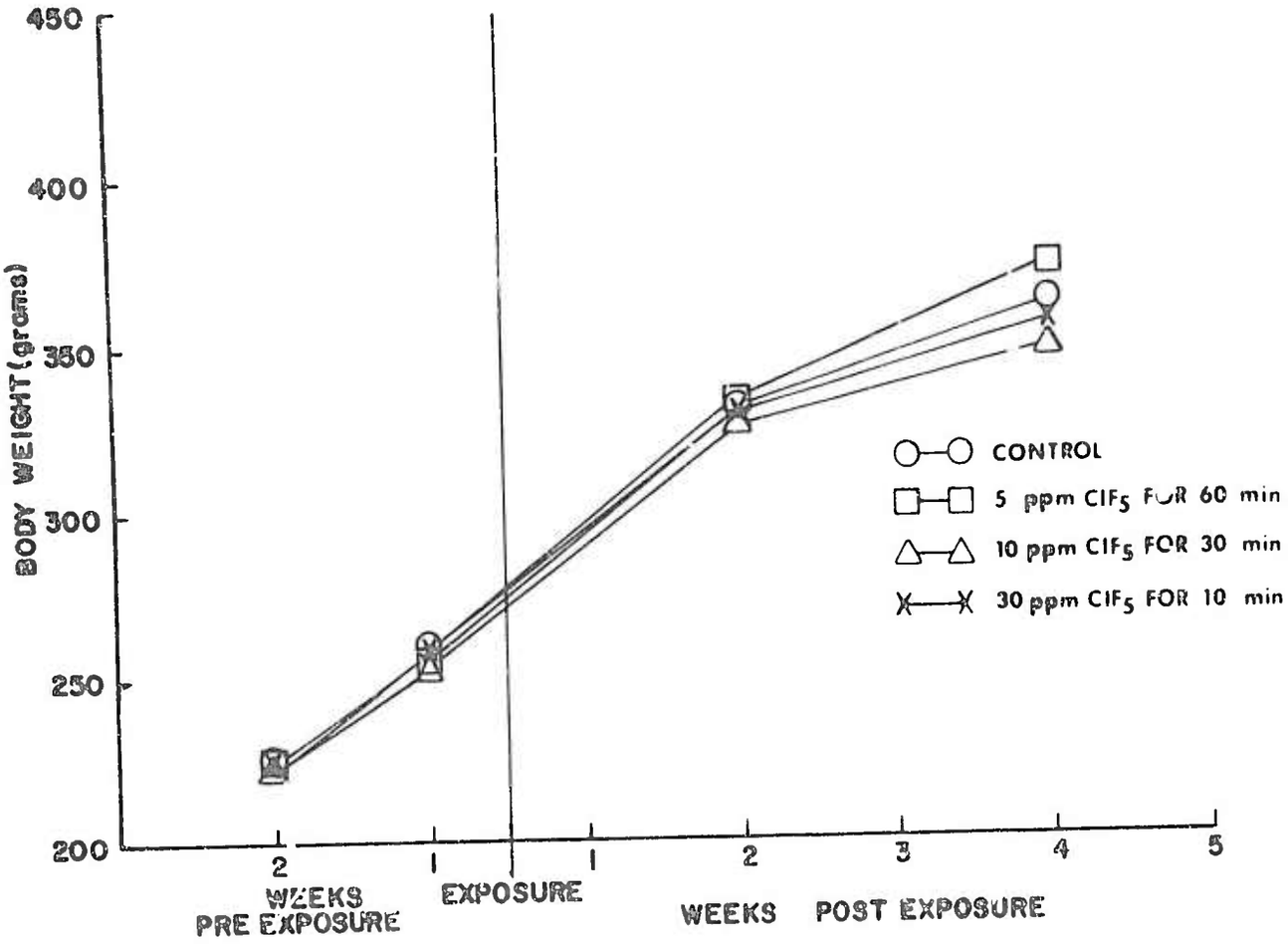


Figure 24. Effect of ClF5 EEL exposures on body weight of rats.

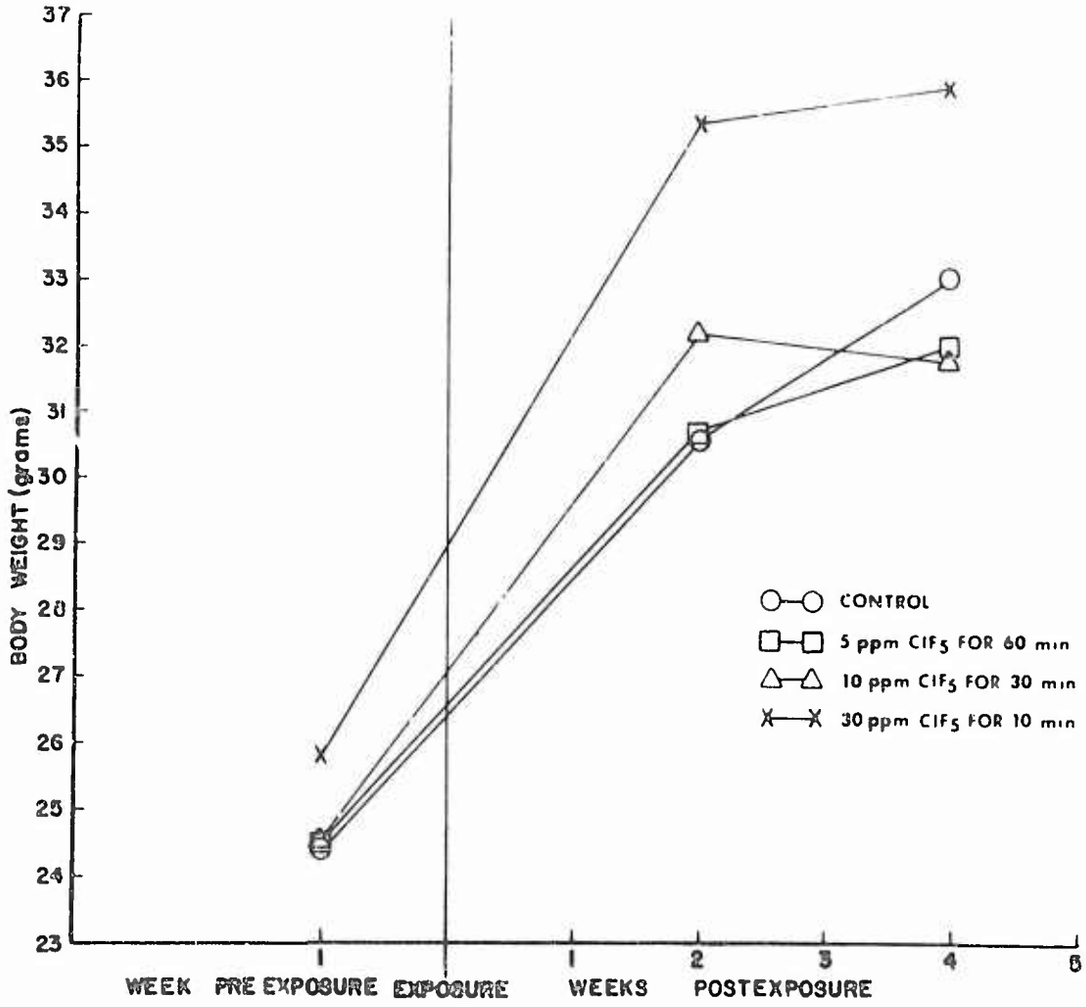


Figure 25. Effect of ClF_5 EEL exposures on body weight of mice.

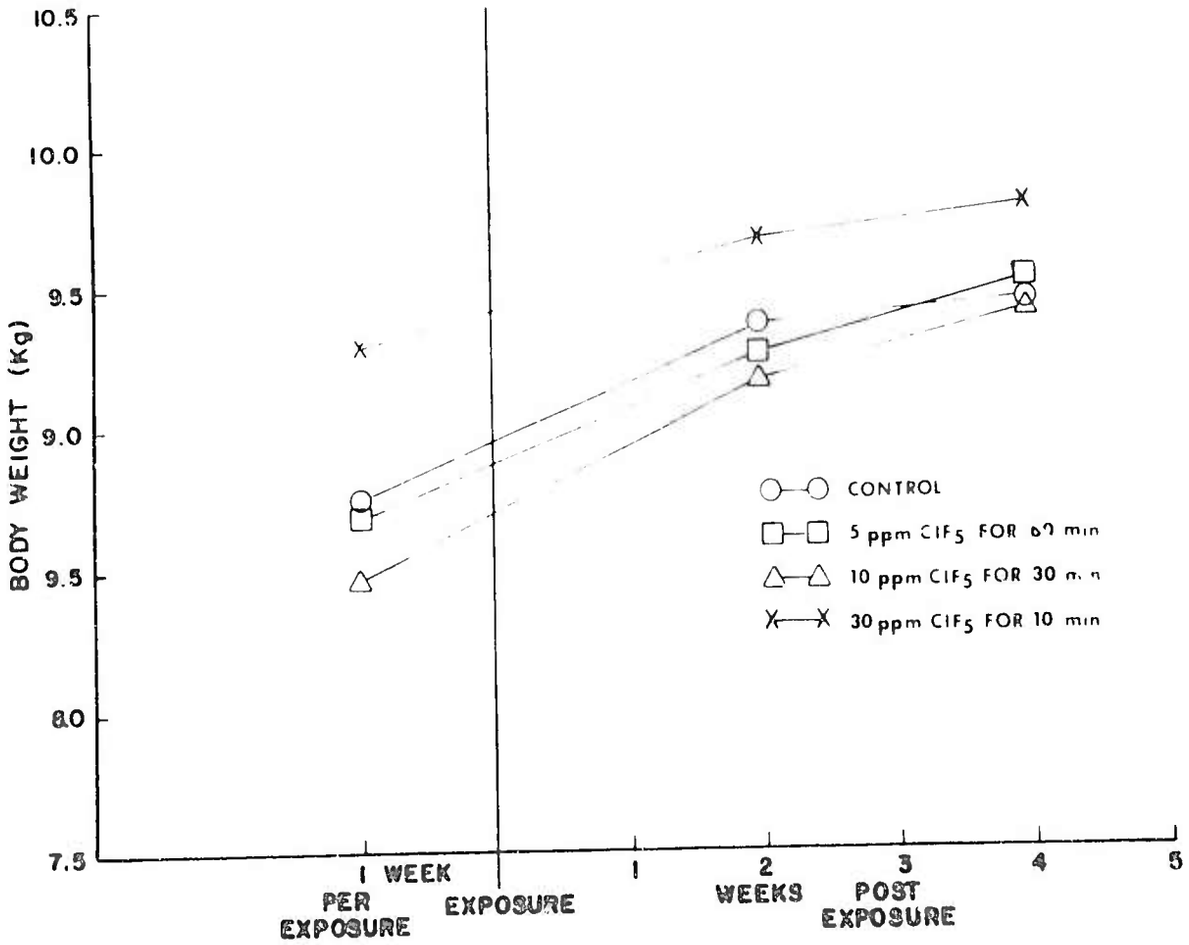


Figure 26. Effect of ClF_5 EEL exposures on body weight of dogs.

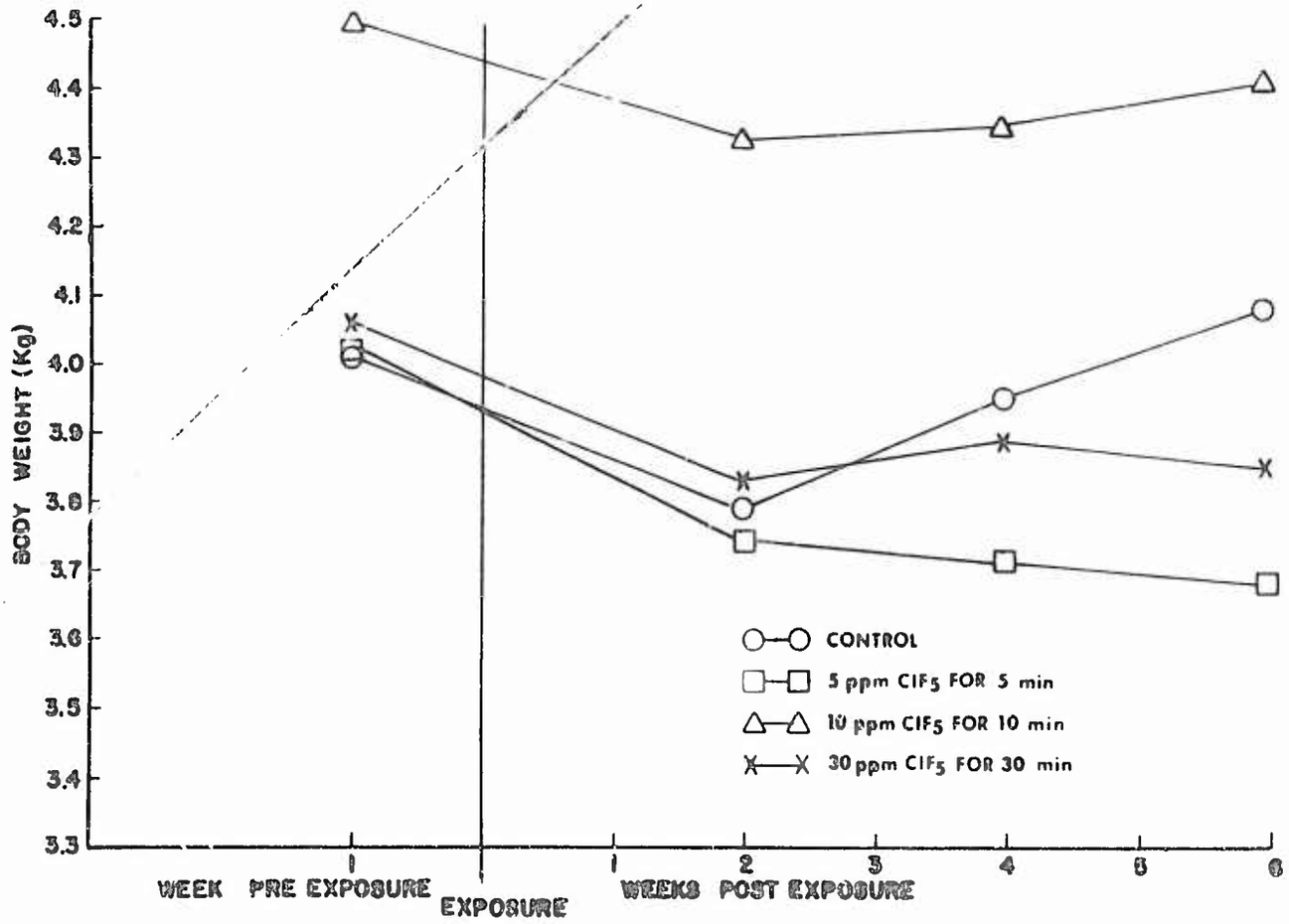


Figure 27. Effect of ClF_5 EEL exposures on body weight of monkeys.

Examination of blood chemistry data for dogs and monkeys revealed scattered instances of significant differences between exposed and control groups, but there was no pattern to these differences, and no trend towards a dose-response could be found.

To further clarify the results presented above, an exposure involving rats and mice only was conducted. Ten mice and 10 rats were exposed to 30 ppm ClF_5 for 10 minutes, with a like number serving as controls. Both species were observed for symptomatology during exposure, and both species were sacrificed immediately postexposure. The mice were examined for gross pathology only, and the rats were used to determine any presence of edema in the lungs. This was accomplished by surgically removing the lungs and bronchial tubes and tying them off 2 cm above the bifurcation to the lungs. The lungs were then weighed on a balance to obtain the wet weight, dried in an oven at 110 C for 24 hours, and reweighed to obtain the dry weight. Significant differences between exposed and control groups would indicate presence of edema fluid in the lungs of the exposed group.

The typical irritation effects of ClF_5 were again observed during exposure in the rats and mice.

Gross examination of mice immediately following exposure showed mild congestion in the lungs of the exposed group compared to controls, with no other gross evidence of damage. Lung weight data for the rats is presented in Table 24. There was a difference of more than 0.1 grams between exposed and control wet lung weights, while the dry lung weights were essentially

identical. This indicates that there was more fluid in the exposed lungs, which means that this level of exposure produces a measurable edema in the rat lungs. There was a significant difference, at the 0.01 level, in grams of water per gram lung weight between exposed and control rats.

Also, there was a significant difference, at the 0.01 level, in lung moisture/body weight ratios with the controls having a ratio of 0.442×10^{-2} and exposed having a ratio of 0.509×10^{-2}. This represented a difference of about 9.5% more water in the lungs of exposed rats compared to controls, and was equivalent to about a pint of water in human lungs. However, judging from the 4-week postexposure observation of other rats exposed to this same level of ClF_5, even this rather severe change in lung condition was not enough to cause significant permanent damage, and in fact the total effect was not enough to induce a change in the rat body weight pattern.

TABLE 24. WET AND DRY LUNG WEIGHTS OF RATS EXPOSED TO 30 PPM ClF_5 FOR 10 MINUTES (N = 10)

	Mean Wet Weight (grams)	Mean Dry Weight (grams)	Mean Grams H_2O/Gram Body Wt. ($\times 10^{-2}$)
Control	1.3941	0.3705	0.4418
Exposed	1.4976	0.3733	0.5087*

*Different from controls at 0.01 level of significance.

In spite of the fact that a 300 ppm-minute dose level does not cause lasting injury, even at the histopathological level, the degree of discomfort experienced by the experimental animals during exposure, and the fact that significant edema resulted in the lungs of rats exposed to this dose makes this level unacceptable as an EEL.

To examine lower levels of ClF_5 which would be acceptable as EELs, preliminary exposures to lower concentrations were conducted. Rats only were exposed to 3 ppm and 7 ppm ClF_5 for 10 minutes. There were 20 rats exposed at each level, 10 for determination of lung weights immediately following exposure (as described above), and 10 to be followed for 28 days post-exposure for body weight patterns and gross and histopathology at the end of that period. Two control groups of 10 rats each were treated in the same manner for comparison with the exposed groups. These exposures were conducted in a specially constructed plexiglass chamber shown schematically in Figure 28. This chamber had a volume of approximately 120 liters and was equipped with a sliding cage drawer to allow for rapid insertion and removal of the animals from the ClF_5 atmosphere. Ambient air from the laboratory was blown through this chamber by an electric blower unit at the rate of 30 cfm. This air was shown to have approximately the same relative humidity as that used in the Rochester chambers in previous ClF_5 exposures, so that any reaction products would be expected to be the same. The chamber was equipped with air diffuser units on each end and was shown experimentally

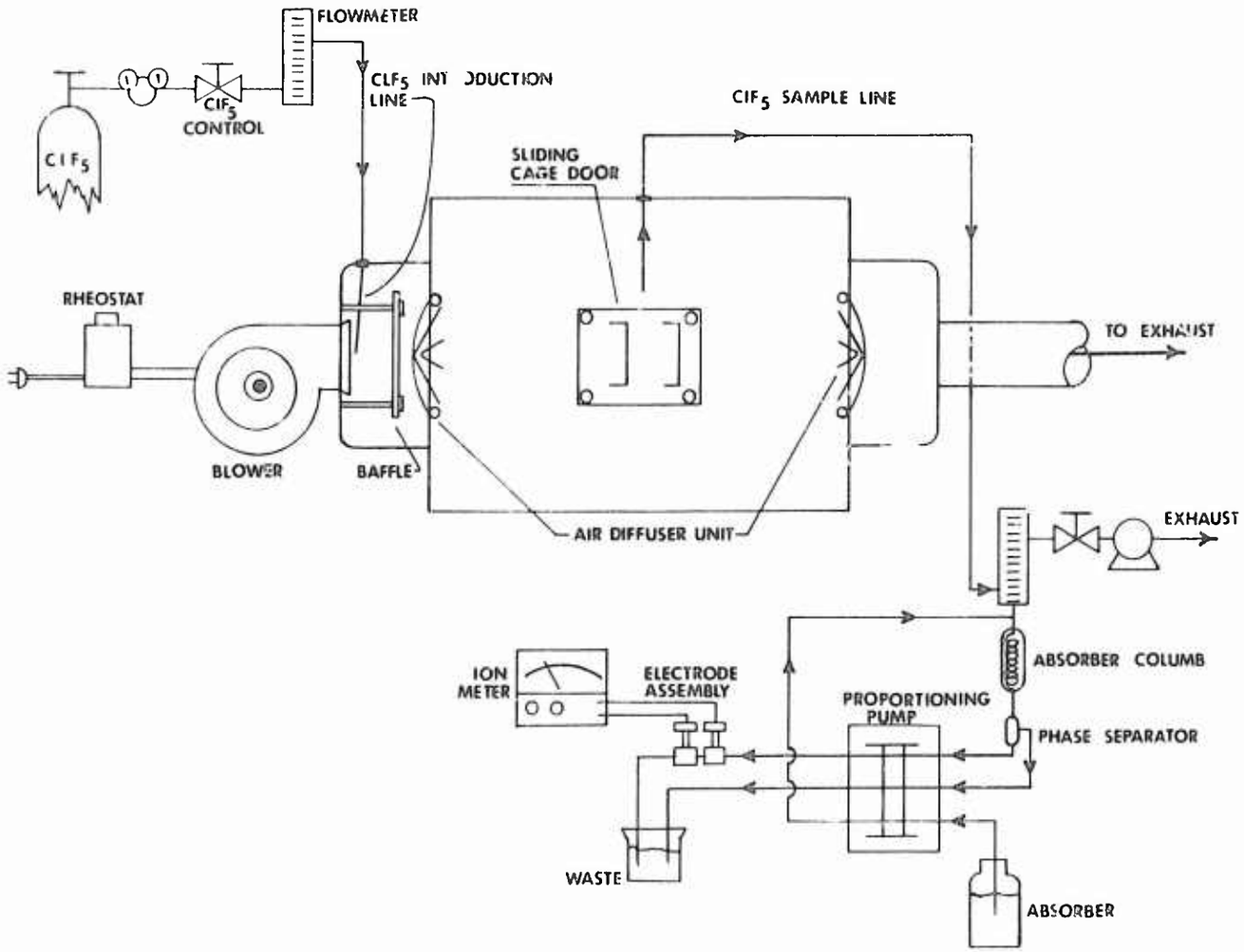


Figure 28. Schematic diagram of plastic chamber for ClF_5 exposures.

to give excellent distribution throughout the entire chamber so that ClF_5 mixture with the carrier air was virtually complete and uniform. The entire chamber was placed in a fume hood, and the exhaust line was routed through a scrubber column to prevent contamination of the atmosphere with ClF_5 fumes.

The ClF_5 was introduced into this chamber at the point of entry of the air input into the diffuser box to insure best distribution. The concentration of ClF_5 in the chamber was monitored continuously during all exposures, with the sample line being placed adjacent to the exposure cage (as shown in Figure 28).

At 3 ppm ClF_5 for 10 minutes exposure there were no visible signs of irritation and there was no increase in lung water. At 7 ppm ClF_5 there was only slight moistening of the rats eyes after 10 minutes exposure. Again no increase in lung water. In both exposure trials weight gain was unaffected.

There are no human exposure data available on ClF_5, but in our work with the compound, there have been occasions when ClF_5 vapors have been inhaled by the staff conducting the exposures. On one occasion, a concentration of 30 ppm ClF_5 was established in a Rochester chamber and one of the rodent cage drawer covers was loosened to allow a "whiffing" of this concentration of ClF_5. One of the staff members experienced headache, mild nausea, unpleasant taste and a mild "burning" in the lungs following

only one good deep inhalation of this level of ClF_5. Fortunately, it seems that the discomfort level for ClF_5 is achieved at concentrations far below those which cause lasting toxicological effects. The greatest problem in setting a realistic EEL level lies in the fact that lacrimation, gagging and burning of moist tissues occurs rapidly and at extremely low levels of ClF_5. These actions may well prevent the self rescue of personnel caught in an accidental exposure situation. Although the low levels themselves appear to be relatively safe from a toxicity viewpoint, the symptomatic effects could sufficiently incapacitate an individual and result in prolonged exposures which would have significant toxicological consequences.

In view of the experimental evidence the tentative selection of EEL values for ClF_5 was judicious and has at least a two-fold safety factor for the most sensitive effect of severe irritation to the respiratory system and the eye.

SECTION III

FACILITIES

The support activities of the THRU essential to the operation of a research activity are usually not of sufficient magnitude to merit separate technical reports. Therefore, these activities are grouped together under the general heading "Facilities" to describe their contributions to the overall mission of the laboratory. Included herein are special projects in analytical chemistry, training programs and engineering modifications to the physical research facilities.

Engineering Programs

The comprehensive preventive maintenance program in action during the past three years has been highly effective, resulting in no experimental time loss due to equipment malfunctions.

Analysis of preventive maintenance records is continuing and provides information on potential equipment failure. The data are evaluated to determine if preventive maintenance schedules are properly selected to provide maximum equipment utilization before component failure and are conducted with maximum efficiency. This approach has shifted expenditures from emergency repairs to preventive maintenance with an overall result of improved operation at no increase in cost. Current efforts in this area are directed towards writing procedures for items of facility equipment requiring preventive maintenance and insuring standardization of these procedures.

Engineering modifications described in this section of the report have been made to upgrade experimental equipment, eliminate safety hazards, and simplify operational factors. Analysis of incidents requiring corrective maintenance have revealed several areas of repeated breakdown. A comprehensive review of the causes of breakdowns has been the basis for adding a new area of responsibility to the engineering functions of THRU. To the responsibility for electrical, mechanical and electronic engineering has been added human factors engineering. This requires an analysis of the man-machine interface for operating equipment to provide workable equipment having maximum safety and reliability features while minimizing maintenance requirements.

Ambient Laboratory Control Panel

The ambient laboratory control panel originally installed included several functions which due to periodic modifications to the ambient chambers had become obsolete. A complete redesign of the panel to fit existing requirements was therefore initiated. Obsolete functions were deleted and additional functions were added as necessary. Several items such as alarm systems, power wiring to chamber blowers and panel layout were refined.

As shown in Figure 29, the existing panel consisted of an upper and lower section. The upper section contained a diagnostic alarm system for system malfunctions. This system was discarded and a scam system, compatible with other alarm systems utilized in the facility was installed.

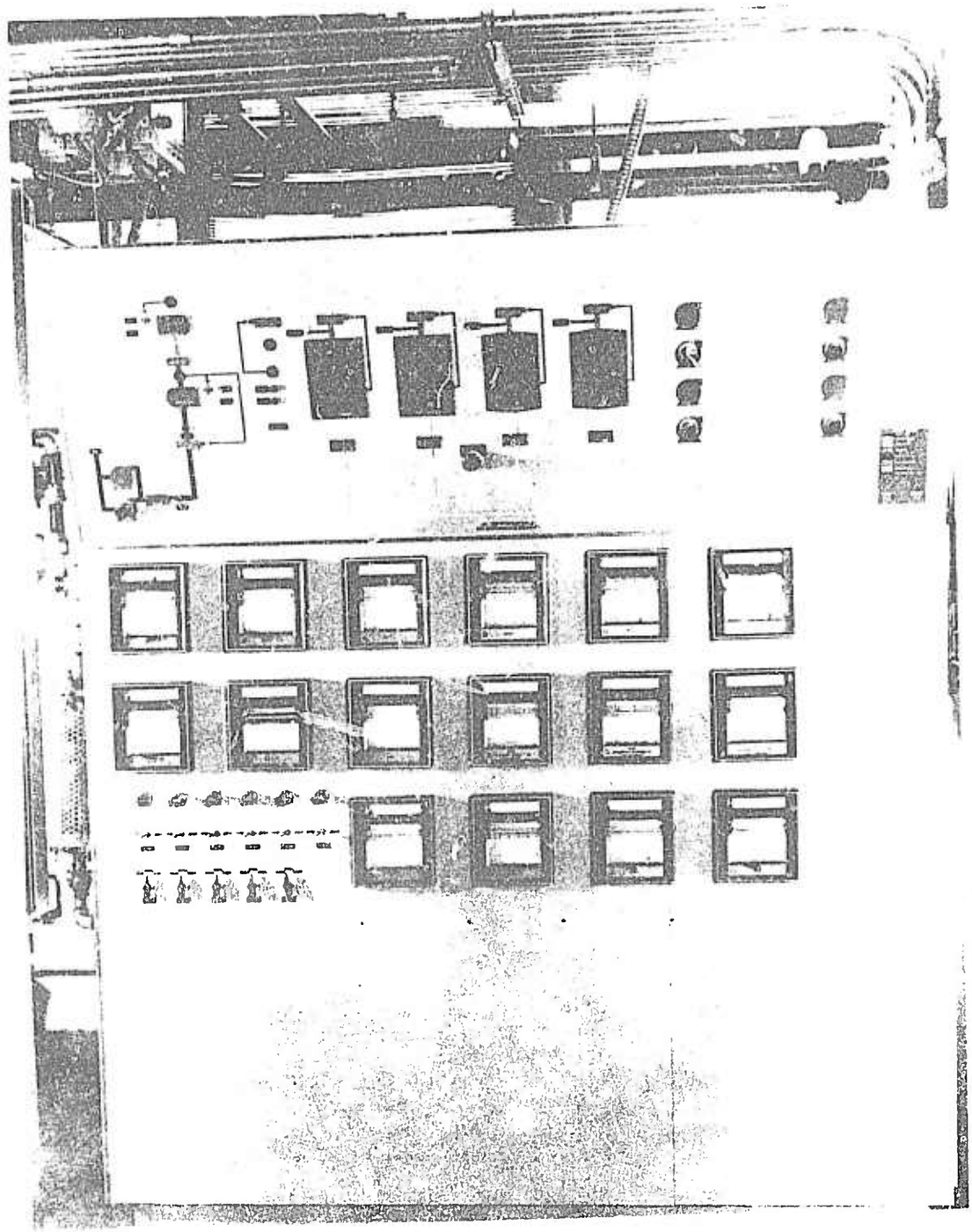


Figure 29. Existing ambient laboratory control panel.

The points covered on the new alarm system are as follows:

a. Alarm points for low flow to chambers A, B, C and D.

b. High differential pressure across the input air filter.

c. High preheat temperature.

d. Low reheat temperature.

e. High reheat temperature.

f. High recool temperature.

These points are connected through the normal scam-type alarm system to the facility main alarm system. In addition an override switch is installed to bypass the main alarm system when routine operations such as chamber cleaning would activate the main alarm system unnecessarily.

The lower half of the existing panel contained recorders and controllers for control of the chamber input air flows and conditioning of the chamber input air. These controllers included those for conditioning outside air to 72 F temperature and 50% relative humidity, monitoring dry bulb temperature to each chamber, monitoring and controlling air flows to each chamber and monitoring wet bulb temperatures to each chamber. The controllers utilized for conditioning outside air were retained. No significant modification of their function was involved other than relocation on the new panel. Due to the monitoring of input air parameters of temperature and relative humidity it was deemed unnecessarily repetitive to monitor these parameters individually for each chamber. These recorders were deleted from the new panel. The units used for recording and controlling air flows to each chamber were retained.

Individual power switches for each recorder were installed directly below each unit. The center portion of the panel contains appropriate switches for control of the input heaters and of each chamber exhaust blower. The completed new panel is shown in Figure 30.

In conjunction with installation of the modified panel, all wiring associated with the chamber operations was rerouted to a new master junction box. This box is equipped with terminal junctions for all wiring and in addition, several safety interlock relays for equipment operation. All obsolete wiring and conduits were removed. The new master junction box is shown in Figure 31.

Contaminant System Modification - Facility A

The existing contaminant vent system for Facility A consisted of a gravity system connected to each of the dome contaminant vents. This type of system presents several operating disadvantages as follows:

a. Slow removal of contaminants.

b. Possibility of contaminant buildup.

c. No dilution of contaminant effluent.

d. Contaminant venting must be at positive pressure.

As shown in Figure 32, a 2" stainless steel pipe was installed around the periphery of the Facility A basement. Each of the 4 contaminant introduction ports for Domes 1 through 4 are located at adjacent points along the pipe. This line is then routed out of the basement and then to a blower located in the mezzanine above the shop. From there, the pipe is then routed through the roof. The

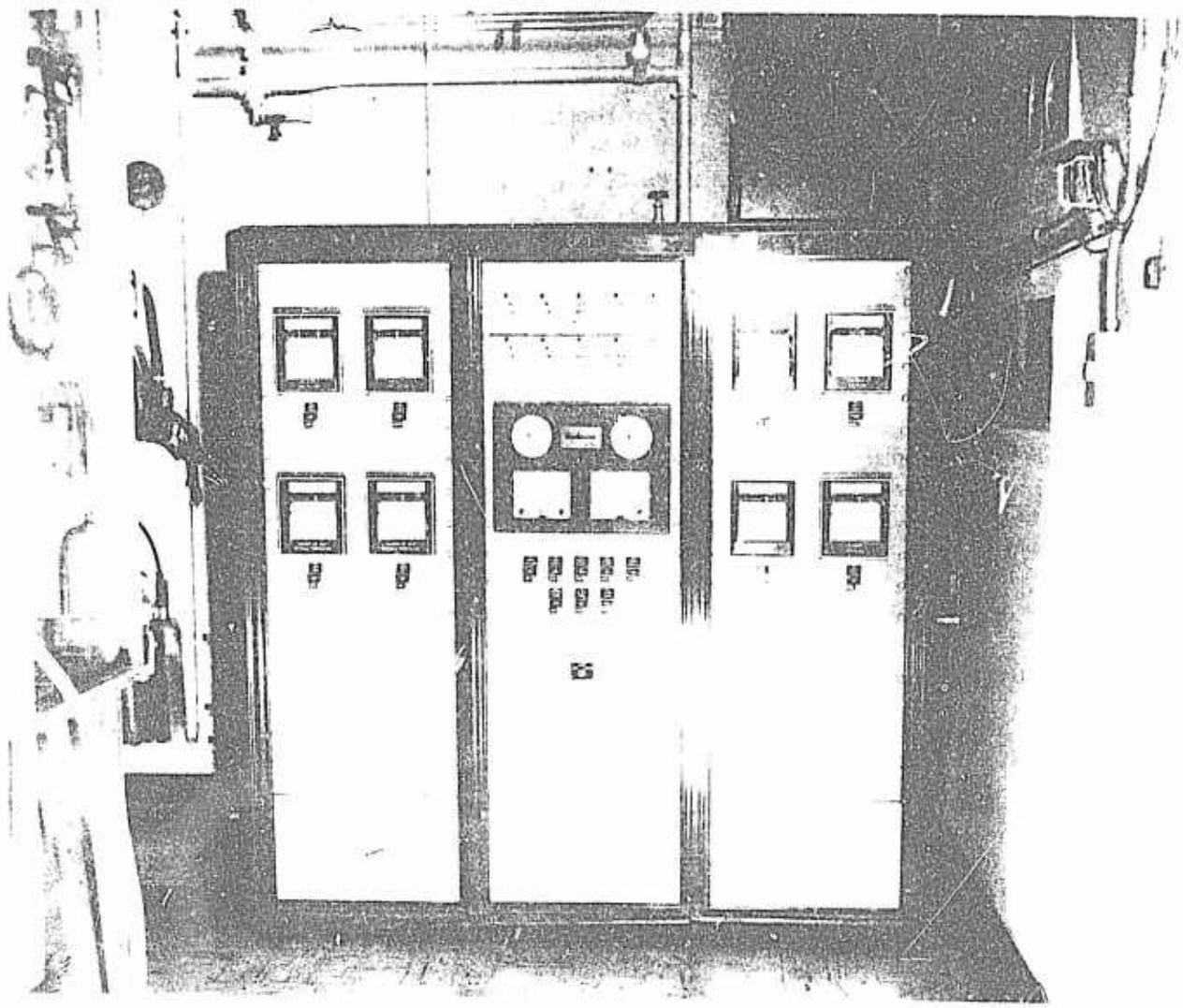


Figure 30. Modified ambient laboratory control panel.

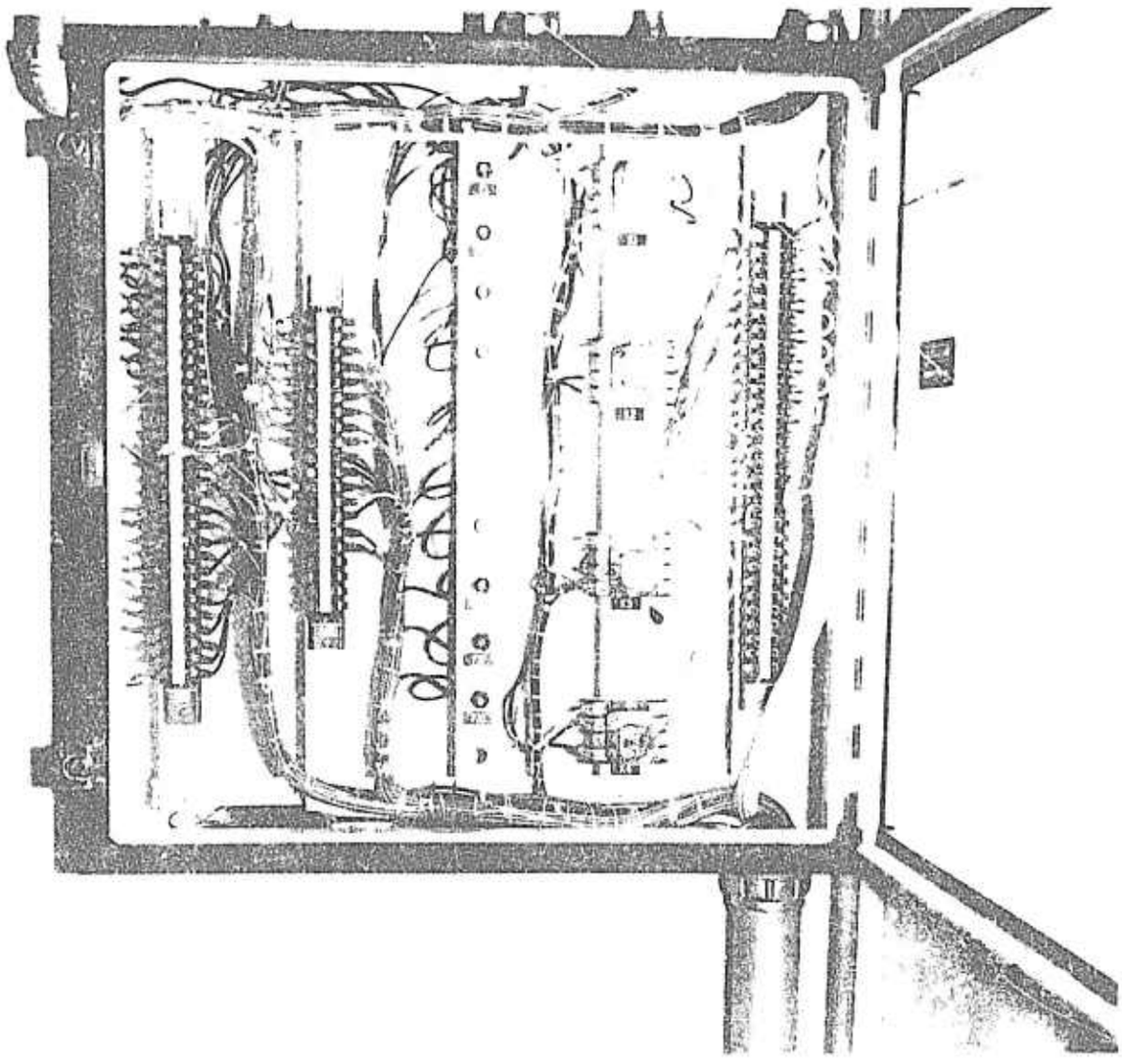


Figure 51. Ambient laboratory master junction box.

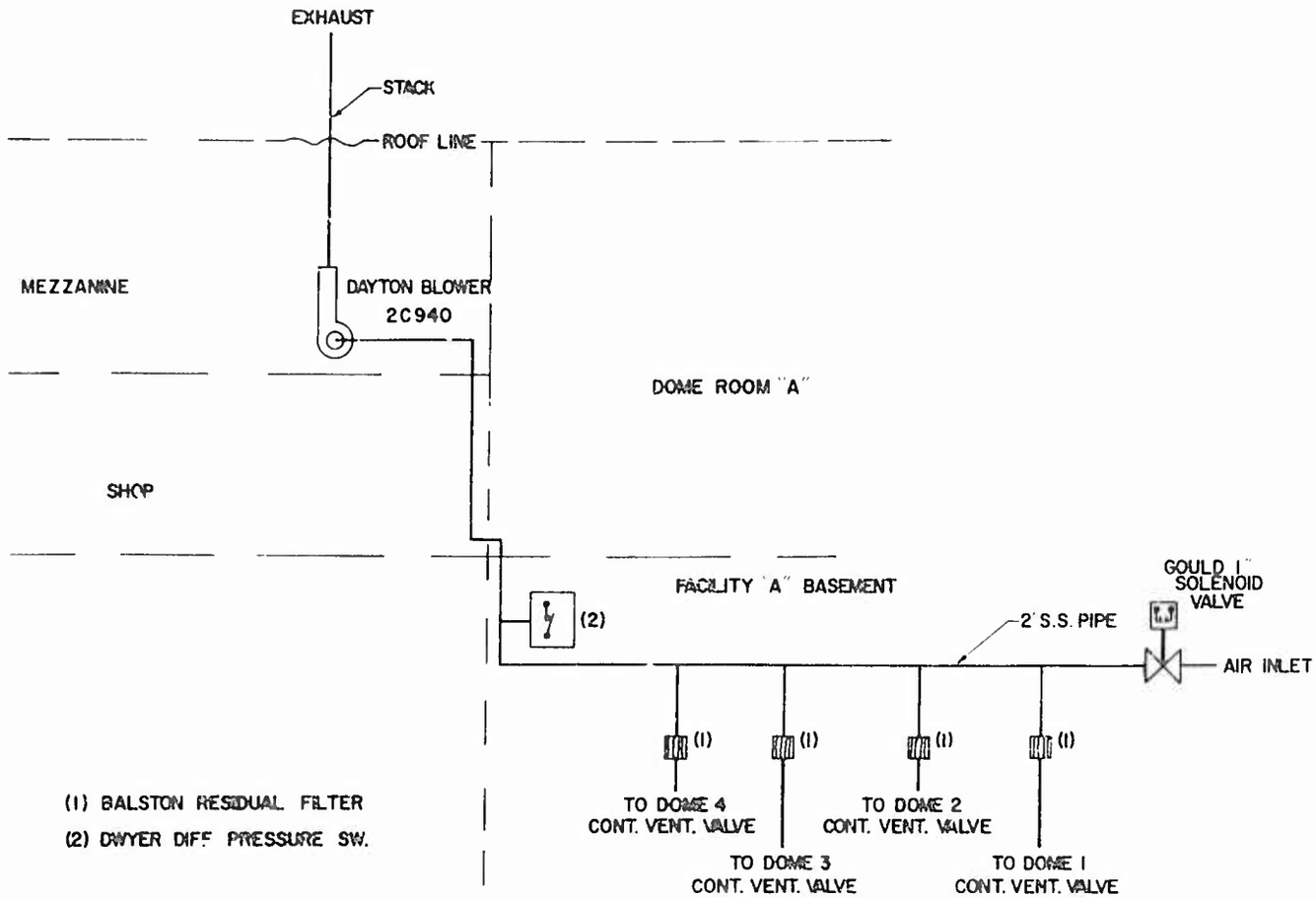


Figure 32. Contaminant vent system - Facility A.

exhaust pipe is terminated approximately 84" above the roofline for effective dispersal of the effluent gases.

There are two safety features installed in the system. The first involves a pressure switch connected to the system to sense negative pressure in the system. If the blower fails, this switch will activate the facility main alarm system indicating contaminant blower failure. In addition, an electrical solenoid installed at the end of the contaminant vent line will close preventing loss of contaminant gases to an occupied area.

Facility Instrument Air System Modification

Instrument air requirements of experiments being conducted in the ambient facility indicated insufficient flow capacities. Evaluation of the problem revealed that an excessive pressure drop was being encountered in the main supply line from the facility air compressors to the laboratories. The size of this line was 3/4" copper tube. Typical pressure drops encountered with experiments being conducted were approximately 25 psi to 30 psi below normal line pressure of 90 psi. The size of the line required to minimize the resultant pressure drop on the basis of the experimental flows contemplated was calculated to be one and one half inches in diameter. An increase in line size was effected from the air receiver tank of the facility air compressors to the main air distribution point for the laboratories involved. This line supplies 90-100 psig air to the ambient laboratory, Facility A laboratory and the Facility B laboratory. A layout of the modified system is shown in Figure 33. In conjunction with the accomplishment of the change

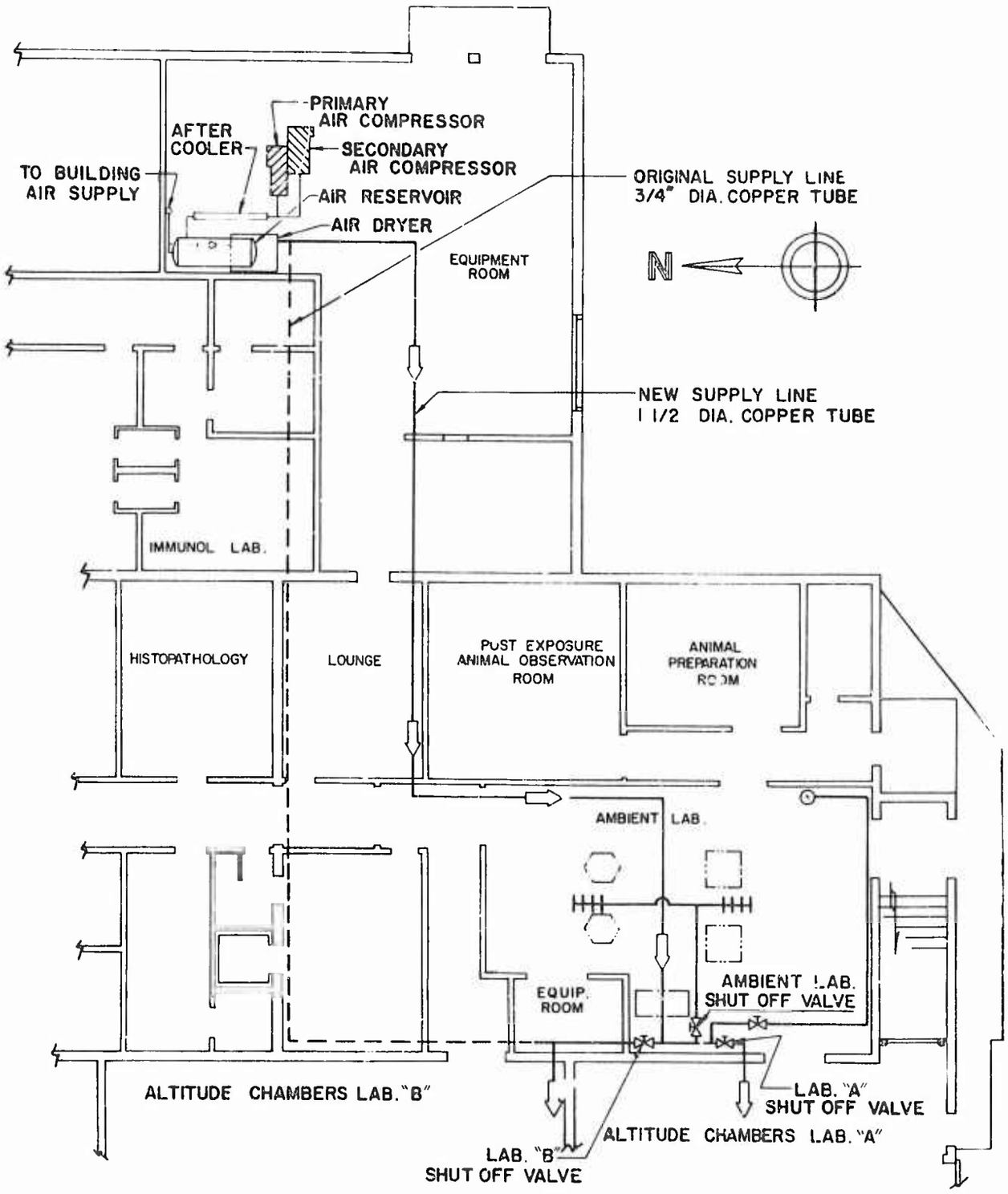


Figure 33. Facility instrument air system modification.

in line size, the air distribution piping was redesigned to provide separate valving to each of the major laboratories involved. This enables each laboratory to be shut off independently so that the air supply can be removed individually without affecting the experimental activities in operation in the remaining laboratories.

Service Walkway - Facility A

A service walkway was designed, fabricated and installed in the Facility A High Bay area. Periodic difficulties had been encountered with servicing various items of equipment installed at or near the ceiling. The main problem associated with this area was physical access to the ceiling lights with reasonable safety. Heighth of the ceiling in this room is approximately 25 feet. Various designs were considered to provide versatility and also safe usage. One difficulty to be overcome was an existing bridge crane used for raising the Thomas Dome tops. It was desired to have access to the complete ceiling area of this laboratory.

A system utilizing an aluminum stage extending the width of the room, traveling on two parallel rails was selected. Special steel rails were designed to be installed at the ceiling to run parallel the length of the room. Vertical support rails were also designed of Unistrut material. An aluminum stage, commercially available, 28" wide and 32' long was selected for the walkway. This stage also included footrails and guard rails. Maximum load rating of the stage was 2000 pounds.

The system consists of five major sections as follows:

a. Left ceiling rail

b. Right ceiling rail

c. Aluminum stage with rails

d. Left vertical stage support

e. Right vertical stage support.

This system was installed and functions as designed. It provides a safe convenient work platform for all areas of the high bay area. The service walkway is shown in Figure 34. It has been utilized by personnel for the maintenance of the fluorescent lighting system in the area. Other items of equipment which may be serviced from this platform are the Thomas Dome bridge crane, the fire sensing equipment and the fire sprinkling system.

Oxygen Breathing System Modification

A two-part project consisting of replacing all parts of the Facility B system and modifying the Facility A system for supplying the systems with air was completed.

The first project consisted of completely replacing all lines and fittings of the Facility B system to obtain leakproof operation. The system was completed and pressure tested and found to be leakproof.

Modification of the Facility A system was completed and provides system operation with either air or oxygen. To accomplish this, the breathing lines in Facility A were required to be separated from the main oxygen supply lines to

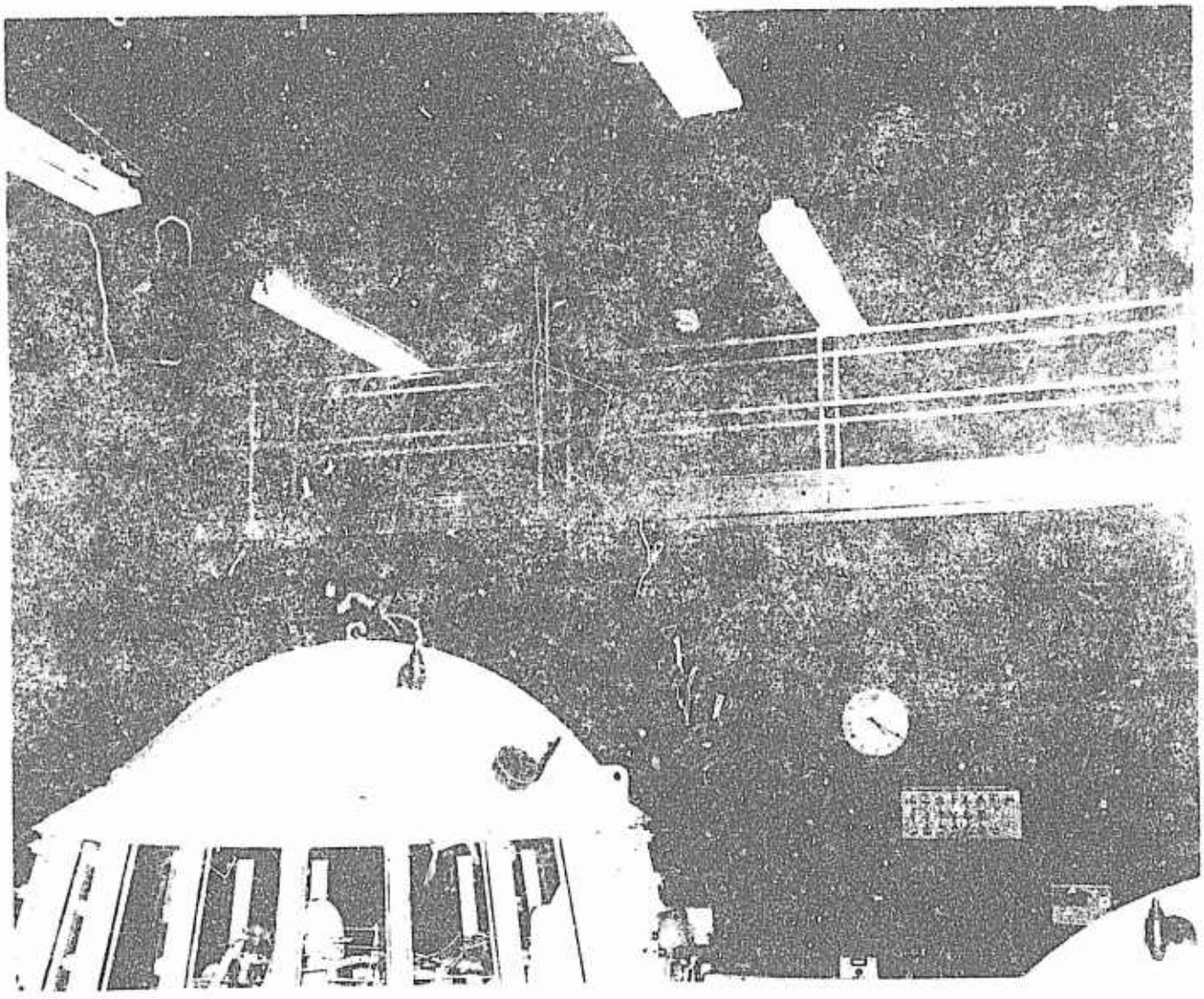


Figure 54. Service Walkway - Facility A.

the domes. Accordingly, the connections to individual dome breathing apparatus from the main line were disconnected and capped. Separate lines were run to each dome and fed from a central air supply point. Design of the interconnecting systems provides normal operation of the breathing systems of both Facility A and Facility B from the facility compressed air system and both automatic change-over to compressed air bottles or oxygen in case of primary system failure. Layout of this system is shown in Figure 35.

Contaminant Vent System Condensate Traps

The normal venting of contaminant gases through the contaminant venting systems resulted in condensation of gases in the vent lines with the consequent collection of contaminant in the lines. Glass condensate traps were installed at each contaminant introduction point preceding the contaminant vent lines. Any condensation of contaminant gases now collects in the glass reservoir of the condensate trap. The trap has an easily removed bowl for ease in cleaning of collected materials. Figure 36 shows a typical condensate trap installation.

Catalytic Heater Test System

Engineering support was provided for a test of a catalytic heater. Parameters tested included atmospheric changes during operation of the heater in an enclosed area. The heater selected for testing was a self-contained model including provisions for propane tanks, mechanical ignition and valve controls. Dome 1 was selected to conduct the test as the monitoring equipment associated with that dome was utilized for analysis of the atmosphere during the test. All

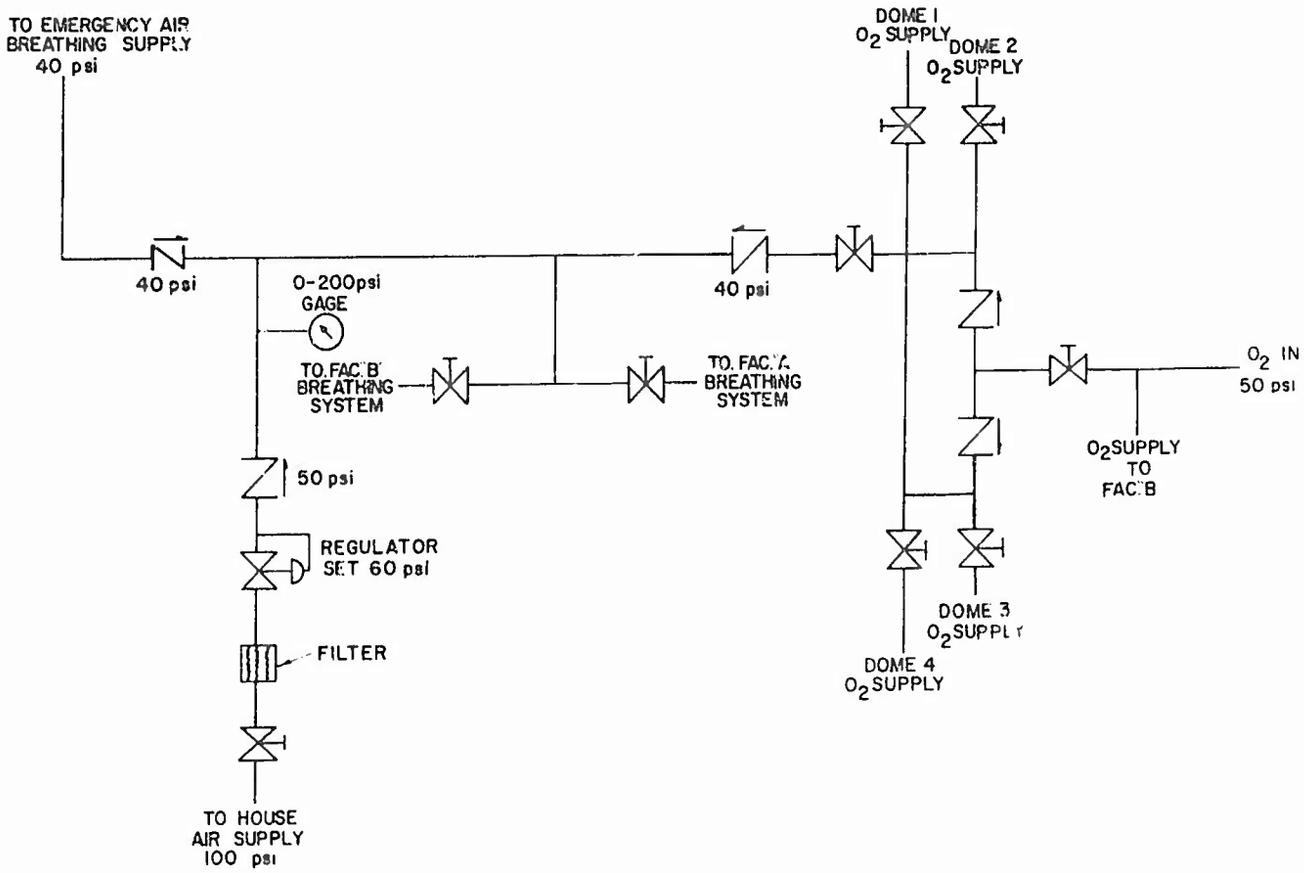


Figure 35. Oxygen breathing system modifications.

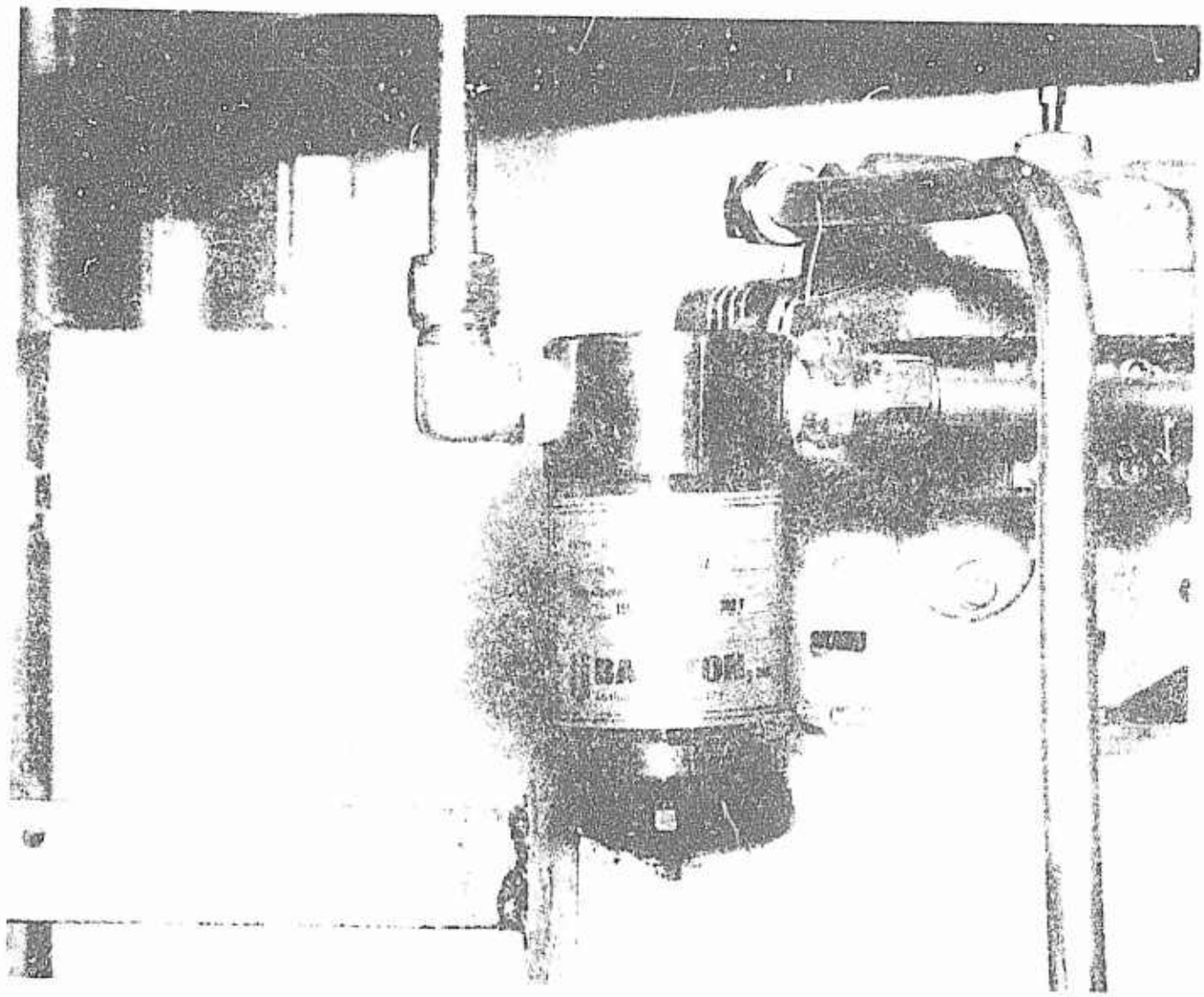


Figure 36. Contaminant vent system condensate traps.

operating controls of the unit were modified to provide ignition from outside of the chamber. An electrical solenoid controlled from outside of the chamber was installed on the unit to activate the mechanical igniter. A temperature sensor was installed near the heater pad to sense heater ignition. This sensor was connected to a master control valve in the propane supply line. Failure of the heater flame automatically shuts off flow of propane to the heater. This control circuit was designed to require manual resetting of the system, preventing inadvertent ignition in case of component failure. The CO_2 analyzers normally utilized in Dome 1 for other systems were recalibrated to readout appropriate CO_2 levels. The O_2 analyzer was recalibrated to analyze O_2 concentrations below 20%. Layout of the system is shown in Figure 37.

Analytical Chemistry Programs

The chemistry department of the THRU performs the routine tasks of monitoring animal exposure chamber concentrations and special analyses on biological samples for non-routine clinical chemistry such as contaminant levels in tissue (skin, lung or blood). Continuous monitoring methods of analysis are frequently not available for the materials subjected to toxicity investigations. Therefore, considerable effort is expended in the development and modification of analytical methods. These projects are the subject of this portion of the annual report.

Analysis of Propane Heater Combustion Products

Portable propane burners, made by Winchester Corporation, have been proposed as heaters for rescue rafts suitable for use in polar seas. The Toxic Hazards

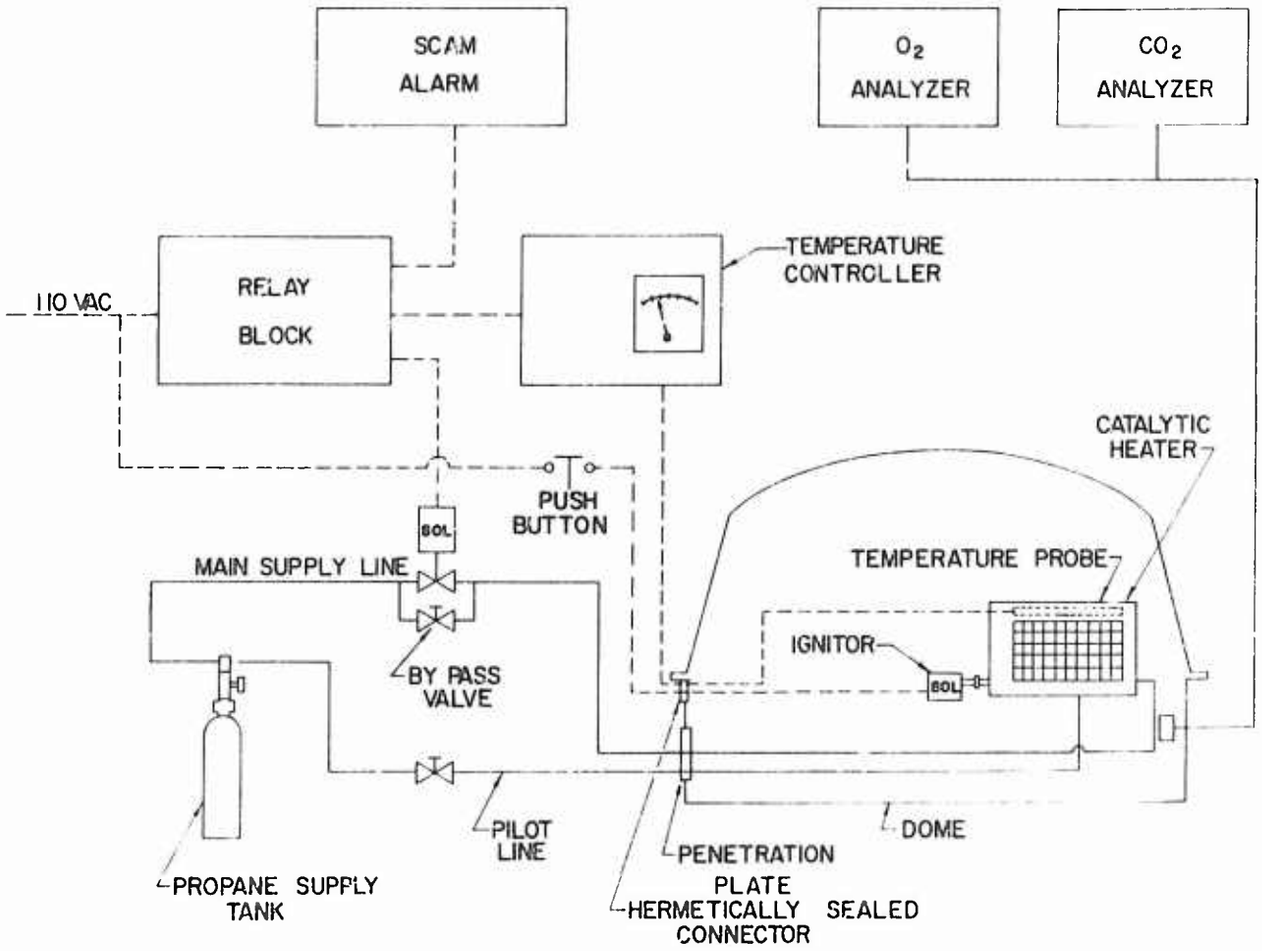


Figure 37. Catalytic heater test layout.

Research Unit was requested to operate one of the units in a Thomas Dome chamber and to measure the consumption of oxygen (O_2) and production of carbon dioxide (CO_2) and carbon monoxide (CO). The resulting data could then be used to predict atmospheric changes in the raft under various conditions. Three different dome situations were used to simulate the possible raft conditions.

1. The isolated chamber was completely sealed at ambient pressure with no air flow. The burner was operated, changing tanks when necessary, until the O_2 partial pressure was insufficient to support combustion. This experiment simulated operation in a completely sealed raft with no ventilation.

2. The exterior dive valve of the isolated chamber was opened and the burner operated until it went out due to lack of O_2. This simulated operation in a raft with limited ventilation.

3. The burner was operated in a dome with an air flow required to maintain the CO_2 concentration at 1%. This experiment verified the ventilation conditions necessary to keep the concentration of this gas in the raft at an acceptable level.

The gas supply and ignition assemblies of the burner were modified so that tanks could be changed and the flame ignited from outside the dome. This required lengthening the fuel lines and adding a solenoid operator to the ignition system. No other changes were made in the system - in particular, the valve supplied with the burner was retained to control the propane supply. CO and CO_2 were analyzed continuously using separate nondispersive infrared analyzers specific for these

gases. O_2 was determined by means of commercially available paramagnetic sensors. Where necessary, the airflow through the experimental chambers was determined through use of a differential pressure flow transducer which is built into the dome air supply system.

Preliminary experiments had revealed that, when operating with the valve fully open, each propane tank was exhausted in about 4 hours with the test unit. At this use rate, and assuming CO production to be negligible, the following theoretical calculations were made:

Weight of propane/tank = 14.1 oz.

Weight in grams = $\frac{14.1}{16.0} \times 454 = 400$ g.

Number of moles propane/tank = $\frac{400}{44} = 9.09$

$C_3H_8 + 5\,O_2 \longrightarrow 3\,CO_2 + 4\,H_2O$ (Assuming complete combustion)

One tank of propane consumed in 4 hours

Moles of O_2 consumed/tank of propane = $9.09 \times 5 = 45.4$

Moles of CO_2 produced/tank of propane = $9.09 \times 3 = 27.2$

Volume O_2 consumed/tank of propane = $45.4 \times 24.5 = 1110$ liters
= 39.2 cu. ft.

Volume CO_2 produced/tank of propane = $27.2 \times 24.5 = 666$ liters
= 23.6 cu. ft.

Volume of Dome = 870 cu ft.

Percentage O_2 consumed/tank of propane $= \frac{39.2 \times 100}{870} = 4.51\%$

Percentage O_2 consumed/hour $= \frac{4.51}{4} = 1.13\%$

Percentage CO_2 produced/tank of propane $= \frac{23.6}{870} \times 100 = 2.72\%$

Percentage CO_2 produced/hour $= \frac{2.72}{4} = 0.68\%$

Volume CO_2 produced/hour $= \frac{23.6}{4} = 5.9$ cu. ft.

Figures 38 and 39 compare the theoretical O_2 consumption and CO_2 production rates shown above with actual hourly values measured in the dome with the dive valve closed (dome completely sealed) and open (partial ventilation). The open dive valve provides a 4-inch diameter hole in the top of the chamber. It is obvious that there is very little leakage in the sealed dome since experimental data points closely match the theoretical. Some leakage is apparent in the dome with the open dive valve, but even here there is no free exchange of chamber atmosphere with the external environment since loss of O_2 and increase in CO_2 are significant.

In preliminary experiments in the sealed dome, it was ascertained that the burner would not reignite easily after the first cylinder had been exhausted. Therefore, the original cylinder was replaced after 3 hours of operation and the burner then operated until it extinguished, giving a total operation time of about 7 hours.

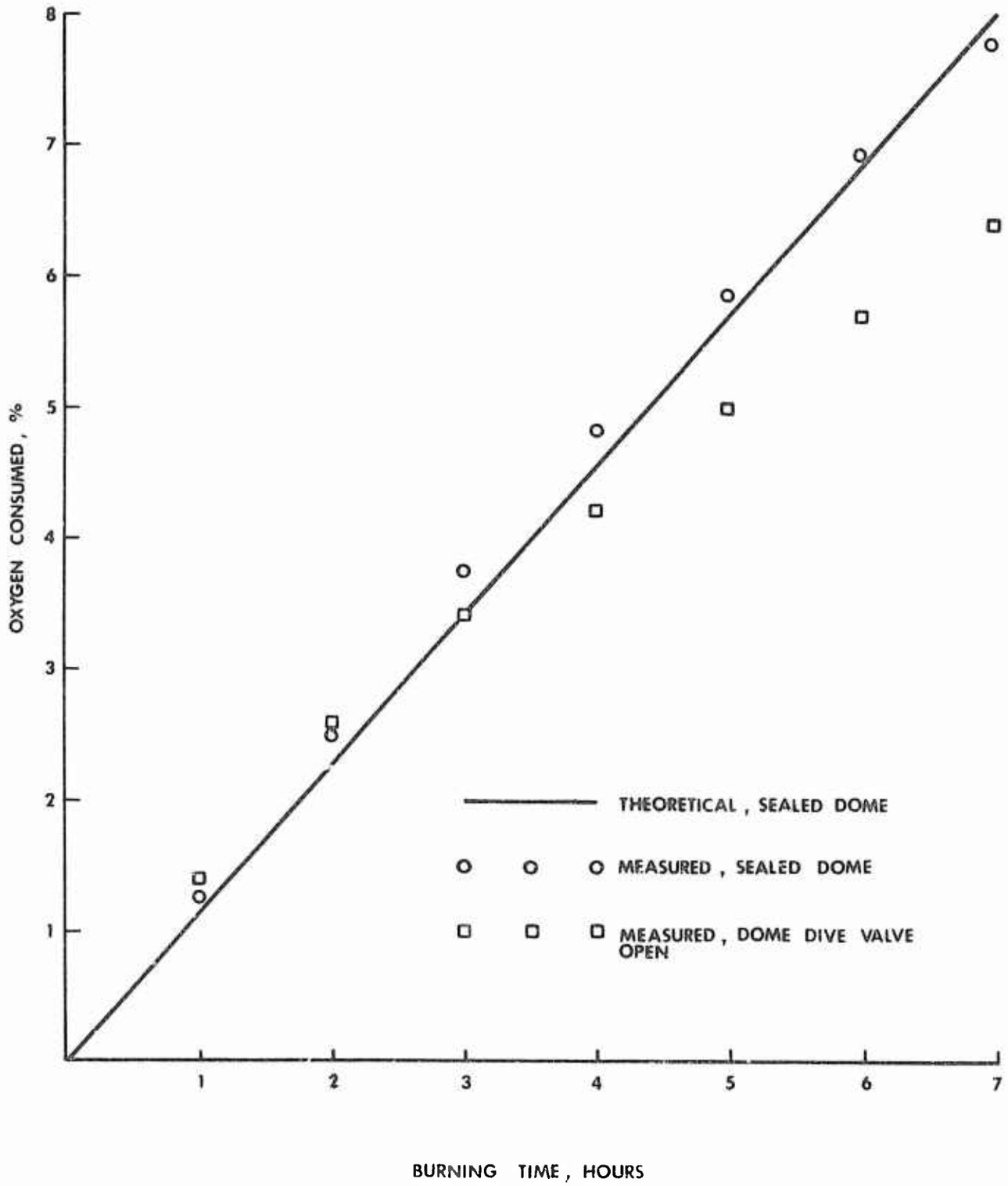


Figure 38. Oxygen consumed by propane heater operation.

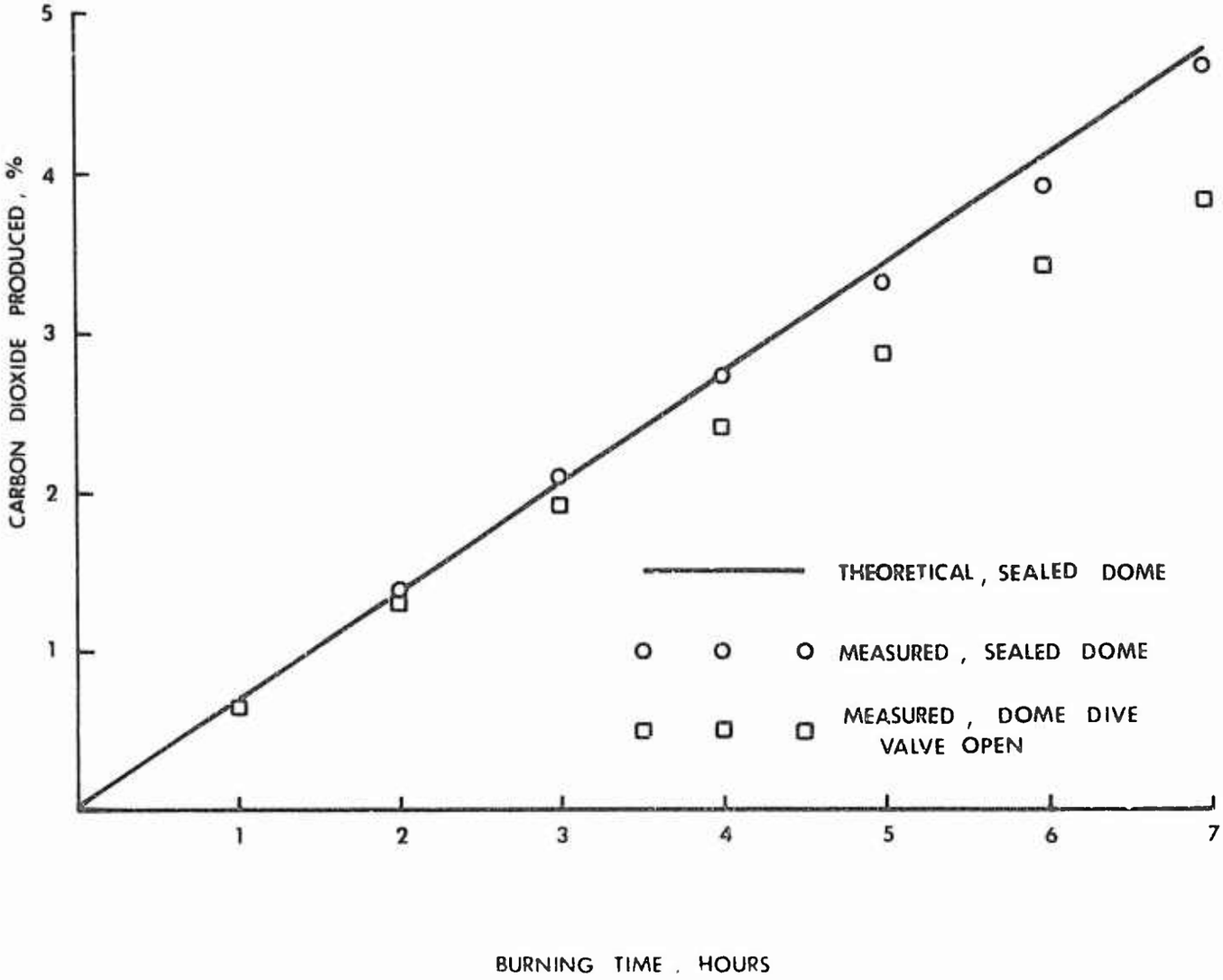


Figure 39. Carbon dioxide produced by propane heater operation.

The highest CO concentration obtained, 95 ppm, occurred in the sealed dome after 7 hours burning. Since this is well below the 250 ppm limit set for human occupation of the raft, it is not the limiting factor for man-rating the system. However, the limit set for CO_2 concentration, 1%, is reached in both sealed and partially ventilated domes after 1-1/2 hours operation. Since both calculation and experiment had shown that 5.9 cubic feet of CO_2 were produced per hour or approximately 0.1 cubic feet per minute (cfm), an air flow of 10 cfm should have yielded an equilibrium concentration of 1%. When the experiment was performed, it was found that an equilibrium CO_2 concentration of 1% was achieved using a nominal air flow of 9 cfm. Since measurements of low flows in our system are not extremely precise, it is quite possible that the flow really was 10 cfm.

With the combustion kinetics of the catalytic heater substantiated, it is possible to make some predictions concerning the raft if the effects of O_2 consumed and CO_2 produced by human respiration are ignored.

1. The critical gas in the system is CO_2 since the highest acceptable concentration of that gas, 1%, is reached before the O_2 level drops or the CO level increases to unacceptable concentrations.

2. Since the raft volume has been calculated to be 64 cubic feet, generation of CO_2 at the rate of 0.1 cfm would produce a 1% CO_2 concentration after 6-1/2 minutes of burner operation in a raft completely isolated from outside air.

3. In order to maintain CO_2 at the 1% level, an air exchange of 10 cfm would be necessary in the raft just as in the experimental chamber assuming a burning rate equivalent to the unit tested.

Comparison of Coal Tar Samples

Two different samples of coal tar were used for the exposures begun May 1972 and those initiated in April 1973. Four drums of coal tar containing light oil were indirectly received from NIOSH for the new series of exposures. Densities and viscosities of the material in each drum were measured and compared with a mixture of 5 parts by volume of coal tar from the previous experiment and 1 part light oil. Results are shown in Table 25. The viscosity was determined by measuring the time of flow of 200 g coal tar from a funnel with a 5 mm aperture.

TABLE 25. PHYSICAL CONSTANTS OF COAL TAR

	Specific Gravity	Viscosity, sec.
Barrel 1	1.115	30.7
Barrel 2	1.117	30.7
Barrel 3	1.121	30.4
Barrel 4	1.121	29.9
Coal Tar + Light Oil	1.107	33.5

When a 600 g sample of the new material was fractionally distilled through a Snyder column, 82 ml of organic liquid and 8 ml of water were distilled below 170 C. Under the same conditions, 90 ml of organic liquid and 15 ml of water were obtained from the mixture made here. Gas chromatography patterns of the new coal tar and the oil mixture disclosed no significant differences. The

fluorescence of the two materials per gram were the same, and about the same percent of solids, 4-4.5%, is obtained from each by heating to 200 C and centrifuging for 10 minutes.

The new material appears to aerosolize more efficiently than the coal tar - light oil mixture used to develop the aerosolization technique. This may occur because there is less water present so that the coal tar - water emulsion is less viscous.

Fluorometric Analysis of Chamber Coal Tar Aerosol Concentration and Tissue Coal Tar Content

The fluorometric method reported by Chambles (1969) for determination of coal tar oil in air samples was adapted to the measurement of coal tar in chamber air and in tissues. The Turner Model 110 Fluorometer using Type 7-60 primary filter and a combination of Type 47B and 2A (also 1% neutral filter at high concentrations) provided sensitivity for measurement in a range from less than 50 ng to more than 30 μg per ml. The basis for measurement is the induced fluorescence of many of the coal tar oils present in the crude tar on activation by ultraviolet light. Standards were made using dilutions in toluene from a gravimetrically determined mass of the same tar being used for the animal exposures.

The methods of analyses are outlined as follows:

A. Chamber Aerosol Concentration

1. The aerosol is sampled on a 47 mm diameter, 0.45μ millipore filter, using a Gelman 1220 filter sample holder, perpendicular to laminar

chamber flow. Sampling time is five minutes, at 5L/min. using a vacuum pump. The flow rate is measured by a flowmeter.

2. The fluorometric zero is set.
3. The filter is placed into 25 ml toluene and swirled briskly for one minute.
4. Fluorometric analysis is completed using the fluorometer and standard curves developed for air sampling.
5. Coal Tar Fluorescent Equivalents are reported in mg/m^3.

B. Tissue Analysis

1. Approximately 200 mg of tissue is weighed to the nearest mg.
2. One hundred times tissue weight in milliliters of toluene is used for extraction (e.g., 213 mg requires 21.3 ml toluene).
3. Tissue is homogenized in toluene with a hand tissue grinder.
4. Mixture is washed twice with 75 ml H_2O in a separatory funnel.
5. Remaining emulsion is broken by shaking. (It has been found that most difficult emulsions may be broken through further dilution with toluene.)
6. Toluene layer is filtered through Whatman-40 paper.
7. Filtrate is centrifuged and supernatant fluorescence read on the fluorometer.
8. Using the standard curves developed for air sampling one obtains $\mu g/ml$.
9. $\mu g/ml$ are then calculated back to coal tar fluorescent equivalents/gram of tissue.

Dilutions of 5.0, 10.0 and 15.0 μg coal tar/ml toluene are made up daily for fluorescence standardization (higher dilutions may be required for low chamber concentrations and tissue analysis). A typical calibration curve is presented in Figure 40. Standard coal tar solutions must be made fresh daily since loss of fluorescence occurs on storage. The fluorometer filters must be kept clean and replaced in the instrument in exactly the same position.

Fractionation of Crude Coal Tar

A part of the responsibility given to the THRU with respect to coal tar was the analytical fractionation of crude coal tar into chemical fractions. This fractionation would be an aid in characterizing coal tar from different sources, but, more importantly, would provide a first step towards isolation of the carcinogenic substances in the crude material.

Most studies of the composition of coal tars relate directly to finding economical use of byproducts of the coking process. The available literature mainly covers separation of materials into acidic, basic and neutral fractions following fractional distillation to relatively narrow boiling ranges. A variety of physical and chemical means are used for further study of these fractions; while pitch, the material remaining beyond the 350 C boiling fraction, which comprises approximately 62% of the original crude tar, is largely uncategorized.

The inhalation toxicology study of coal tar being conducted in this laboratory has aimed at using the whole tar rather than a volatile portion. Preparation of fractions for carcinogenicity characterization required some development, since,

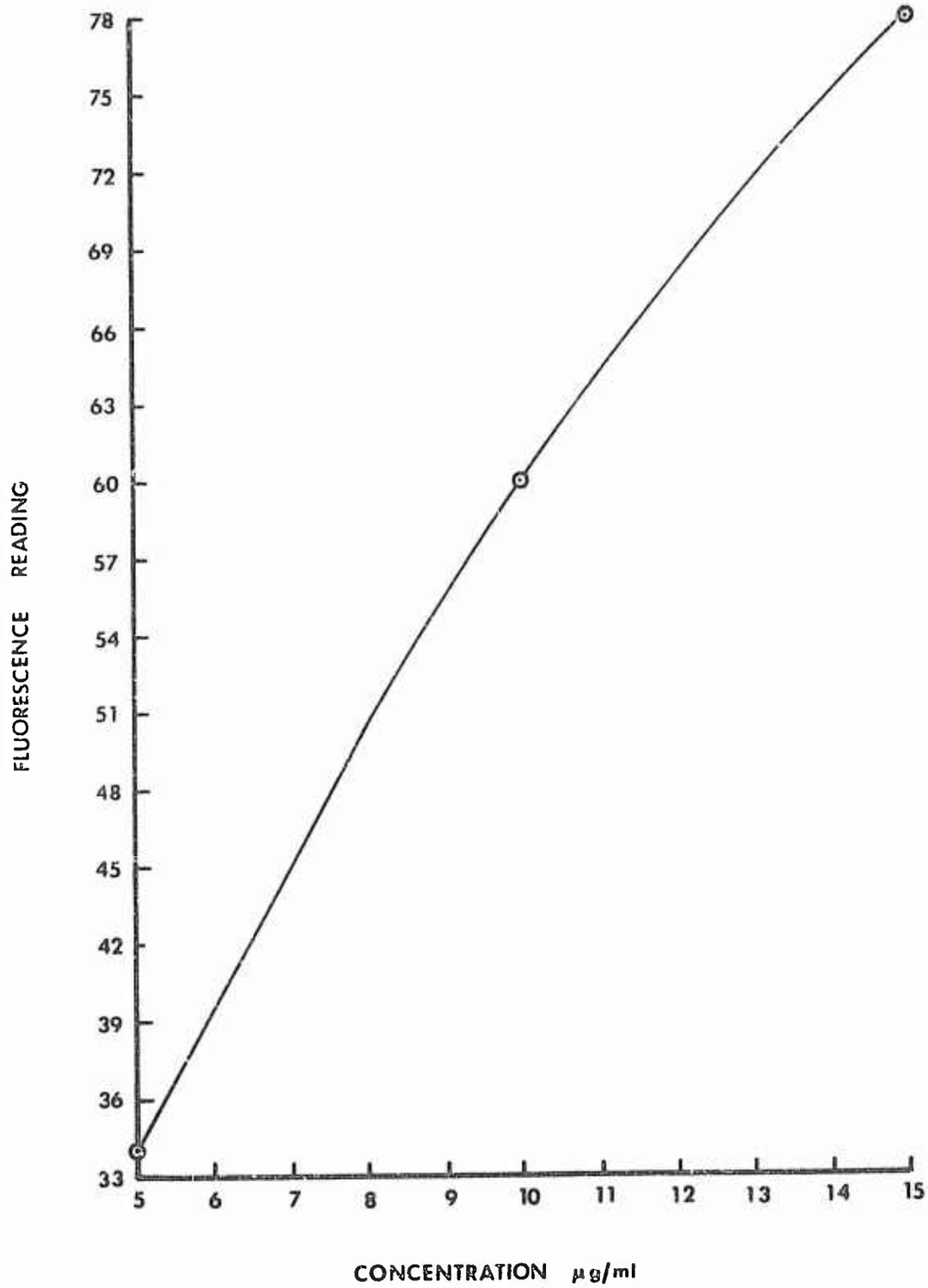


Figure 40. Fluorescence of coal tar in toluene.

as noted above, little or no work has been done on material containing the oven effluent crudes. This report gives an account of the investigation into methods for separation of whole coal tar into acid, basic and neutral fractions and the use of adsorption columns for further separation of the neutral fraction.

In order to accomplish the initial series of animal exposures, it had been necessary to eliminate particulates in coal tar by 1:1 dilution with benzene and centrifugation. The benzene was removed by fractional distillation before chamber use. Initial attempts to extract the crude tar and the tar used in the exposures with aqueous acid and base were unsuccessful because of the formation of refractory emulsions. However, 3:1 dilution with benzene followed by centrifugation removed solids and reduced viscosity sufficiently to permit satisfactory manipulation.

Step 1. Three volumes of benzene were added to 1 volume of coal tar and the solutions centrifuged. Centrifugation was performed using Model PV-110, International Equipment Company with a 242 rotor, in 200 ml glass centrifuge bottles at 2700 RPM. Usually, about 15% by weight of solids was removed by this procedure. The precipitate was washed once in benzene and the benzene then used for dilution of more crude tar.

The benzene insoluble portion was not extensively studied, but when ashed in air, yielded a residue of 0.4% by weight of the sample. No distinguishing IR spectra were obtained from the powder. Some fluorescent material could still be dissolved out readily after four washes in benzene. The dark brown material

produced after Soxhlet extraction of the insoluble portion could be pulverized into a dry powder. The benzene insolubles are called "A" in the general scheme of separation (see Figure 41).

Step 2 - Separation of Tar Acids by Base Extraction. Both the clarified coal tar-benzene mix and a 10% NaOH solution were preheated to 65-70 C. Equal amounts (200 ml each) were swirled for 1 minute in a separatory funnel. Three phases resulted, an aqueous, a benzene and an oily precipitate which appeared mainly at the solution interface.

The benzene and aqueous layers were separated and filtered. The benzene layer was washed 2 times with water which was added to the aqueous portion. The water layer was washed twice with benzene which was added to the benzene fraction. The oily precipitate was washed 2 times with both water and benzene each being returned to proper fractions. The precipitate at this step is identified as "B" in Figure 41. The benzene solution at this point contained tar bases and neutral oils which were held for Step 3.

The aqueous portion, containing sodium salts of tar acids was placed in another separatory funnel and immediately titrated to a drop beyond neutral (p Hydrion paper) with 10% H_2SO_4 solution. A heavy oil was released which slowly settled to the bottom. This heavy oil was then separated and water washed 2 times, the washings being returned to the aqueous portion. The washings were accomplished in a test tube and the oil brought down by centrifugation. This heavy oil (tar acids) is assigned "C" in Figure 41.

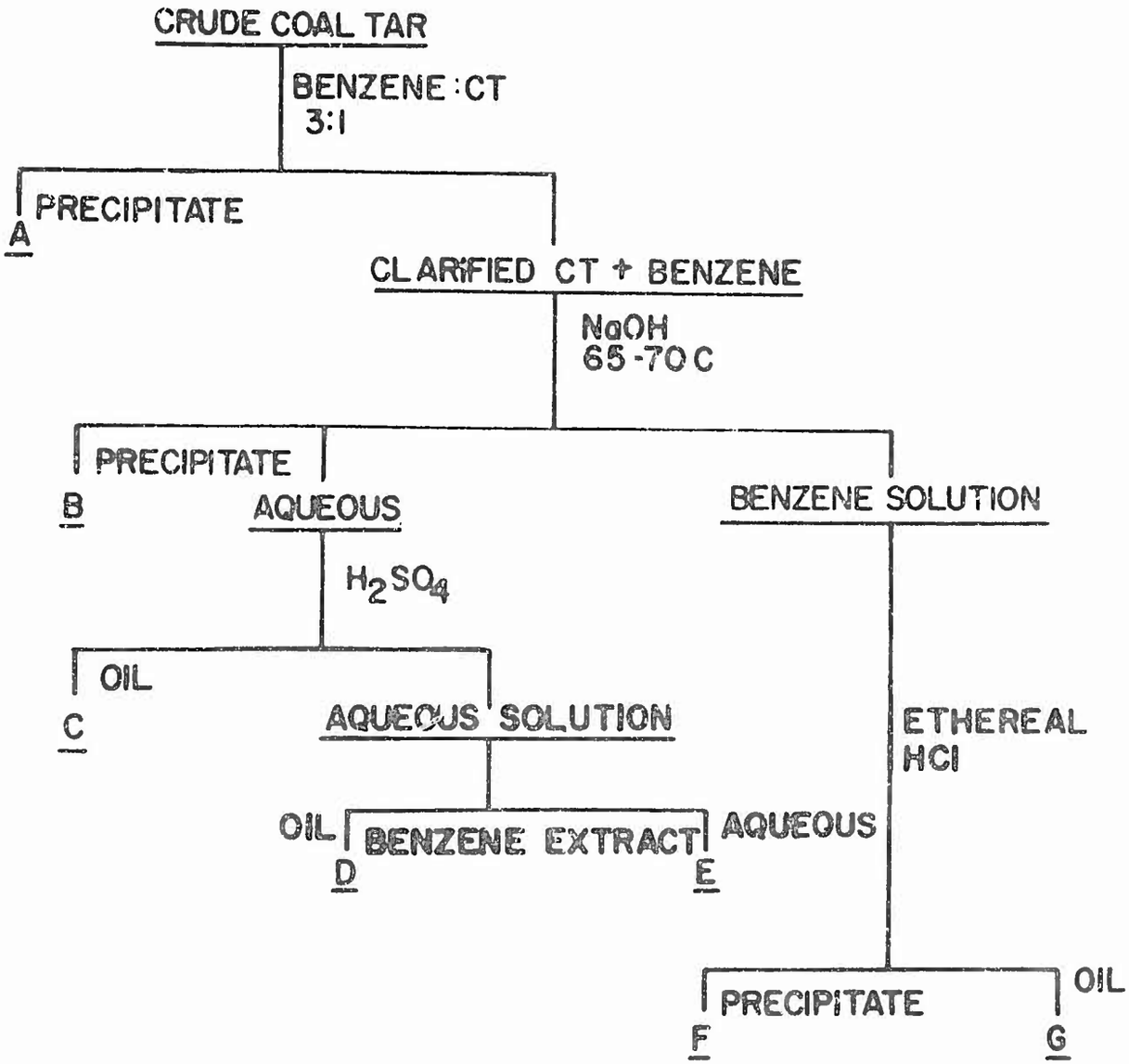


Figure 41. Scheme of separation of crude coal tar to acid, base and neutral fractions.

The neutralized aqueous solution was extracted 3 times with benzene and the combined benzene extract allowed to evaporate at room temperature to constant weight. The oil remaining is "D" and the aqueous portion "E" (Figure 41) remaining contained essentially only sodium sulfate and was discarded. It is expected that fraction "B" will be neutralized and combined with fractions "C" and "D" to give a mixed crude coal acid fraction for animal experimentation.

Step 3 - Separation of Tar Bases by Acid Precipitation. Attempts to extract the remaining coal tar with 10% aqueous H_2SO_4 precipitated large volumes of solids which prevented phase separation, and alternative means of coal tar base separation were investigated. In the most successful technique, the benzene solution resulting from the initial alkaline extraction step was carefully titrated with constant swirling to a drop beyond neutrality (p Hydrion paper) using a concentrated ethereal-HCl solution (approximately 20% gaseous HCl in ethyl ether). Extreme care is required at this step to prevent formation of a black resin-like material. The fine dark powder "F", separated by filtration in the scheme, was washed 2 times with benzene.

Step 4 - Neutral Oil Fraction. The benzene fraction remaining after separation of the bases contained the neutral oils "G". The benzene was allowed to evaporate at room temperature to concentrate this phase.

Step 5 - Column Separation of Neutral Oil Fraction. Solvent elution adsorption chromatography was applied to the neutral oil fraction. Dry packed alumina (Woelm, neutral activity - Grade 1) columns 1" x 16", both activated and 2%

deactivated, were studied. N-pentane, paradioxane and methanol were used as eluents, and in that order. Activation of the alumina was accomplished by heating at 200 C until constant weight was attained, usually overnight. Partial deactivation was achieved by stirring the activated material at ambient humidity until it gained 2% of its dry weight. The material was then stored in sealed jars.

The separation of crude coal tar by the method described above gave six recovered fractions. Table 26 shows the percentage of each collected fraction from Figure 41 in terms of weight of the crude tar.

TABLE 26. IDENTIFICATION AND PROPORTIONS OF COAL TAR FRACTIONS

Type	Fraction	% of Crude Tar by Weight
Benzene Insolubles	A	15
Tar Acids	B (sodium salts)	10
	C (free acids)	3
	D (free acids)	1
Tar Bases	F (hydrochlorides)	18
Neutral Oil	G	70

The infrared absorption spectrum of each fraction was examined using a Beckman IR-5A. The benzene insoluble fraction "A" displayed no significant peaks. Fractions B (when neutralized), C and D each displayed characteristic spectra of phenolics and aromatic acids. "F" appeared to have the scalloped N-H absorption of amine hydrochlorides as well as aromatic characteristics. "G", the neutral oil fraction, showed aliphatic and aromatic type spectra.

The separation of the neutral oil fraction by adsorption chromatography was performed on activated and 2% deactivated alumina. The resulting fractions were observed using IR spectroscopy. The two systems differed basically in the pentane fraction. On partially deactivated alumina evidence is found for aliphatics and aromatics in the pentane fraction whereas on activated alumina, the pentane fraction shows only saturated aliphatics. According to Snyder (1968), the remaining separation is due almost entirely to number of aromatic carbon atoms in the molecules. Table 27 shows the separations by percent of neutral oil in each fraction.

TABLE 27. RESULTS OF ELUTION ADSORPTION CHROMATOGRAPHY

Fraction	2% Deactivated Alumina	Activated Alumina
n-pentane	10%	2%
P-dioxane	85%	95%
methanol	3.5%	1%
residue	1.5%	2%

Thin layer chromatography was performed on the eluent fractions of the neutral oil for comparison of fractions and for possible separation to individual compounds. Fluorescence spot observation was the only means of visualization used successfully. In one separation, over 36 spots were noted following two dimensional developments. Since the identification of specific compounds was not the object of this work, this technique was not pursued further.

Recent work on crude coal tar separation has been directed toward checking the reproducibility of the technique described in this report and in scaling it up to separate large fractions suitable for chronic mouse skin application. In fact, the separation has been found to be reproducible, and separation of a 2 kg sample of crude tar has proceeded through the isolation of all tar acid fractions.

Stability of Monomethylhydrazine (MMH) in Water

A recent paper (Toth & Shimizu, 1973) has indicated that 0.01% MMH in the drinking water of golden hamsters produced malignancies when administered for life. Before an attempt to reproduce this experiment could be made by the THRU, it was necessary to gain some information concerning the stability of MMH in drinking water under various conditions. Accordingly, 2 procedures were developed for the analysis of MMH.

The first technique was gas chromatographic, utilizing the following conditions:

Column: 4' x 1/8" Teflon®

Packing: Chromosorb 103, 60/80 mesh

Carrier gas: Nitrogen at 5 psi.

Sampling is accomplished by drawing 1.0 µl of air into a microsyringe followed by 2.0 µl of water containing MMH. Retention of MMH is about a minute, and the peak area is measured using a mechanical integrator. A typical integrator count for 0.01% MMH was 1900 compared to 90 for pure tap water.

Although the gas chromatographic procedure appeared satisfactory for the analysis of MMH in water, it had certain disadvantages - reproducibility was not as good as desired; the procedure was time consuming; and injection had to be very precise or erroneous results were obtained. In order to overcome these shortcomings, a titration method utilizing iodine was developed in which the MMH solution was titrated with a solution of 0.01N iodine containing 80g/L potassium iodide, 40 g/L disodium phosphate, and 12g/L monopotassium phosphate. There is a sharp yellow to colorless endpoint. The titration and gas chromatographic methods give the same results for MMH concentration whether fresh or after extensive decomposition. However, the titration is much more precise and was therefore the method of choice in following the decomposition of MMH in water.

Preliminary experiments demonstrated that MMH was stable for weeks when prepared in deaerated, distilled water and stored in a stoppered flask under nitrogen. When an air headspace was maintained in the storage bottle, the rate of decomposition was found to be a function of the solution acidity whether distilled or tap water was used. The decrease with time of MMH concentration in tap water under various pH conditions is depicted in Figure 42. Initial air headspace was 60 ml over 400 ml of approximately 0.009% MMH solution and increased to 180 ml as liquid was removed. The losses of MMH are significant in the 8.3 and 6.2 pH solutions, but only 5% after 24 hours in the pH 3.8 solution, and zero in the pH 2.0 solution. The pH's of these solutions were adjusted by addition of concentrated hydrochloric acid (HCl) to the MMH solutions. Previously, it had been determined that the same degree of stability could be conferred on MMH solutions if citric acid were used to

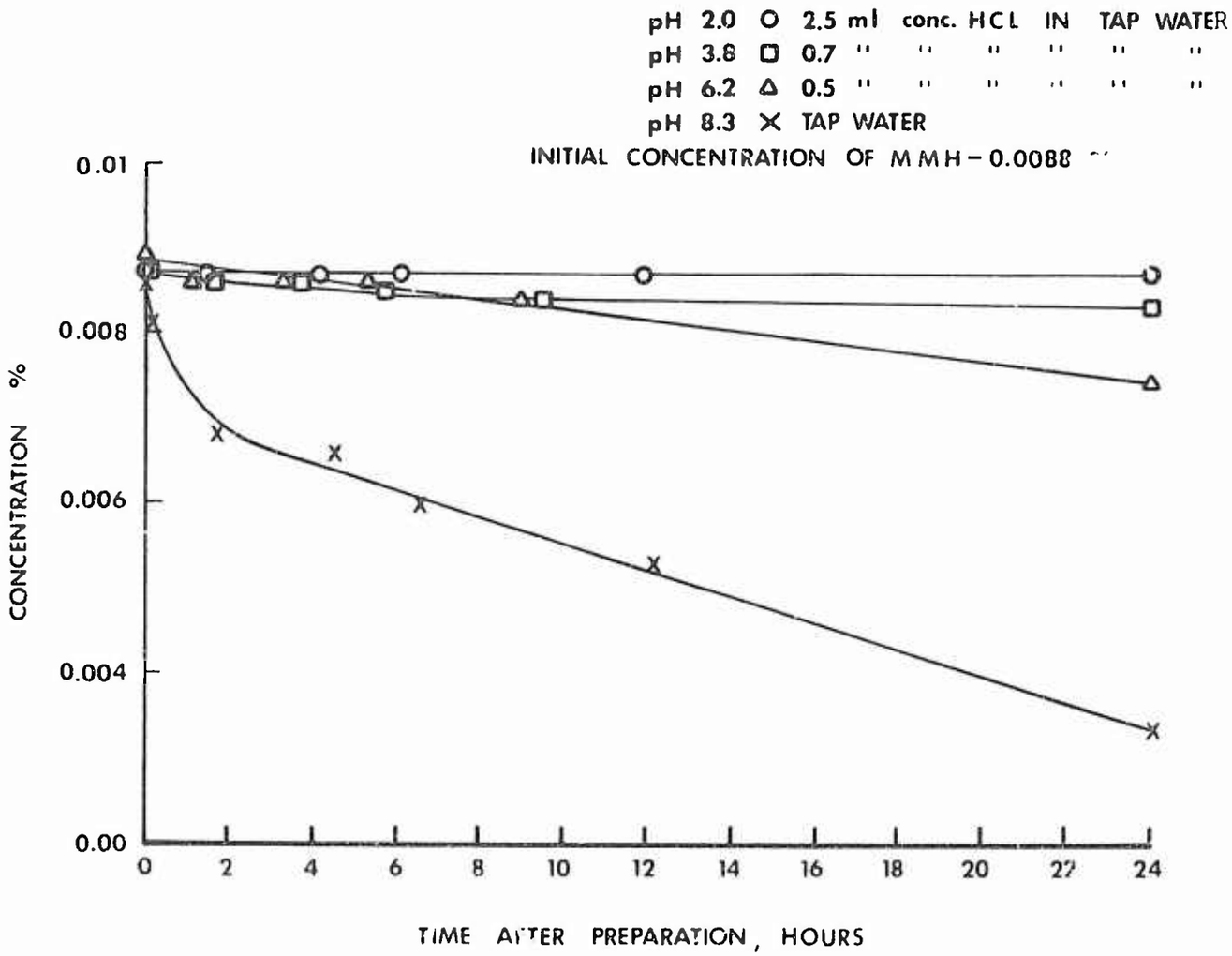


Figure 42. Effect of pH on MMH decomposition in drinking water.

adjust the pH. However, since citric acid appeared to have a deleterious effect on rat weight gain while HCl had none, it was decided to use HCl to adjust the MMH solutions in tap water to a pH of 4.0 At this pH, replicate experiments demonstrated that no more than 5% degradation could be expected. During the experiment, animal water bottles were emptied, rinsed and refilled with MMH solution every 24 hours. Some of the 24-hour solutions were analyzed for MMH content. Results from the first 4 days yielded an average of 3% loss in the solutions adjusted to pH 4.0 and 29% in the bottles to which no acid had been added.

Training Programs

The Laboratory Operations and Animal Care Training Programs described in the 1971 and 1972 annual reports were continued this year as programmed. Phase I and Phase II training cycles were scheduled for all newly hired chamber technicians and those experienced technicians requiring refresher courses. The existing cadre of technicians participates in the on-the-job training of the new personnel.

Monthly Emergency Training Procedures are unannounced deliberate equipment failures or simulated emergencies involving personnel responsible for the operation of the Thomas Domes and the exposure laboratory area. These training episodes insure that the technicians will react with the proper procedure in the event of an actual emergency situation. The actions of the technicians involved in these training exercises are carefully monitored by their supervisors to make sure the proper procedures are adhered to. The following list details the emergency training procedures covered during this reporting period:

Date	Procedure	Personnel Participation*
June 1972	Fire in Airlock with Entrant	ABCD
July 1972	Ambient Blower Failure	A
August 1972	Vacuum Pump Failure	A
September 1972	Air Compressor Failure	A
October 1972	Complete Power Failure	A
November 1972	Rescue of Incapacitated Dome Entrant from Airlock	ABCD
December 1972	Ambient Blower Failure	A
January 1973	Vacuum Pump Failure	A
February 1973	Fire in Dome with Entrant	ABCD
March 1973	Fire in Airlock with Entrant	ABCD
April 1973	Fire in Exposure Laboratory Area During Dome Entry	ABCD
May 1973	Air Compressor Failure	A

*A - Shift Operator

B - Safety Observer B

C - Safety Observer C

D - Dome Entrant

American Association for Laboratory Animal Science (AALAS)

Certification Program

The AALAS educational and certification program for laboratory animal technicians described in last year's annual report was continued during this year.

Several technicians have studied for certification at the next higher level, but personnel changes in the Air Force Veterinary Medicine Branch of AMRL have prevented a qualified examiner from being able to administer the required examinations. A minimum of five technicians are expected to become qualified at the next level during the coming report period.

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